FOURTH REVIEW CONFERENCE OF THE PARTIES TO THE CONVENTION ON THE PROHIBITION OF THE DEVELOPMENT, PRODUCTION AND STOCKPILING OF BACTERIOLOGICAL (BIOLOGICAL) AND TOXIN WEAPONS AND ON THEIR DESTRUCTION

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BACKGROUND PAPER ON NEW SCIENTIFIC AND TECHNOLOGICAL DEVELOPMENTS RELEVANT TO THE CONVENTION ON THE PROHIBITION OF THE DEVELOPMENT, PRODUCTION AND STOCKPILING OF BACTERIOLOGICAL (BIOLOGICAL) AND TOXIN WEAPONS AND ON THEIR DESTRUCTION

Prepared by the Secretariat

1. In paragraph 21 of its report (BWC/CONF.IV/1), the Preparatory Committee for the Fourth Review Conference of the Parties to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction decided to invite States Parties that wished to do so, including the Depositary Governments, to submit to the Secretariat information on new scientific and technological developments relevant to the Convention. This information should cover the applications being made of such developments and their relevance to various aspects of the Convention.

2. The present document contains the information provided by States Parties to the Secretariat, as of 30 October 1996, pursuant to paragraph 21 of the report of the Preparatory Committee.

Cuba

Information for the IV Conference to review the Biological Weapons Convention in connection with the principal scientific and technological developments in Cuba in the past 25 years in the field of microbiology and the areas of veterinary and plant biomedicine and biotechnology - August 1996

The information compiled in this paper cannot be regarded as exhaustive, but reflects in the broadest and most comprehensive manner possible the work accomplished by a group of the most representative institutions in the field in question.

Major results

National Centre for Agricultural Health (CENSA)

* Development of diagnostics for human and veterinary use

* Development of diagnostics for detection of plant pathogens

* Molecular diagnosis of hard-to-detect micro-organisms by conventional means (mycoplasmas, viral diseases of interest in veterinary medicine and bacterial zoonoses)

* Production of vaccines by conventional means and research for the development of recombinant vaccines for veterinary use

* Diagnosis of exotic and quarantinable diseases in animals and plants.

Centre for Genetic Engineering and Biotechnology (CIGB)

* Obtaining, production and use of biological substances: alpha leucocyte and recombinant interferons; recombinant streptokinase; recombinant interleukin 2 (IL-2); recombinant human epidermal growth factor (EGF); transfer factor; human insulin; beta galactosidase enzyme; recombinant human gamma interferon; recombinant protein A; etc.

* Development of technologies for the production of DNA restriction and modification enzymes

* Obtaining of recombinant vaccines against the hepatitis B virus and the tick Boophilus microbius, as well as a natural vaccine against neonatal and post-weaning porcine colibacillosis. Work is under way on possible candidate vaccines against HIV/AIDS

* Obtaining of hybridomas secreting monoclonal antibodies and rat/human hybrid antibodies

* Studies on genetic and immunological characterization of proteins and coatings of micro-organisms

* Development of diagnostics for communicable diseases in humans, animals and plants by conventional methods and by means of molecular biology. Development of new diagnostic studies for SUMA

* Obtaining of transgenic plants and animals (sugar cane, cabbage, potatoes, tobacco, tilapia, rabbits, etc.) and commercial varieties of pest-resistant plants. Technologies for in vitro maturation and fertilization of bovine oocytes

* Studies of infection by hepatitis C, epidemic neuropathy and other diseases

* Production of bioremediators and biological control agents (nematocides).

Centre for Molecular Immunology (CIM)

Obtaining and production of:

* A library of monoclonal antibodies for immunophenotyping of lymphocyte subpopulations

* A library of monoclonal antibodies for immunohistochemistry of tumours

* Injectable monoclonal antibody lor-t3 for prevention and treatment of rejection in organ transplants

* Two monoclonal antibodies for visualization of cancer.

Work is currently under way on new monoclonal antibodies for use in nuclear medicine: to treat lymphomas and lung and breast tumours; recombinant humanized monoclonal antibodies; vaccines for cancer; and basic research on regulation of idiotype networks.

National Centre for Biopreparations (CNB)

Production/research centre which produces 62 types of culture media for fungi and bacteria; recombinant vaccine against hepatitis B (obtained by CIGB); phosphorylation of streptokinase, interferons and albumin; raw material for the biopharmaceutical industry; packaging of products for parenteral use on humans

* Research on introducing new culture media in production processes; development of preparations of allergens for diagnosis and treatment; and development of natural pick-me-ups for human use.

National Centre for Scientific Research (CNIC)

* Use of genetic engineering to develop attenuated strains for immunization against cholera

* DIRAMIC system for rapid detection of urinary infections and the susceptibility of infectious germs to antibiotics.

National Centre for Plant Health (CNSV)

* Use of electron microscopy, serology and molecular biology for diagnosis of phytopathogenic micro-organisms

* Obtaining of antiserums and production of diagnostic kits for plant pathogens

* Development of technologies for the biological production of substances for pest control in cultivated plants

* Use of integrated management techniques for farm pest control

* Development of commercial crops for commercial production of biological substances.

Pedro Kouri Institute of Tropical Medicine (IPK)

* Epidemiological surveillance of exotic communicable diseases. Control measures to prevent their spread in Cuba

* Improvement and automation of the national system for epidemiological surveillance of communicable diseases

* Development of new methods for diagnosis of communicable diseases (amoebas, Fasciola hepatica and dengue). Production of biological reagents and cooperation in the development of new diagnostic kits (SUMA). Introduction of advanced technology (PCR, etc.)

* Participation in preliminary basic research for obtaining viral and bacterial candidate vaccines for humans. Evaluation of new vaccines (against meningitis and hepatitis B). Studies on the impact of vaccines used in the National Vaccination Programme (triple virus - measles, rubella and mumps)

* Research on dengue. Risk factors and technological advances relating to haemorrhagic dengue. Identification of the geographical origin of the Cuban strain of the 1981 epidemic. Basic studies for the purpose of obtaining a vaccine

* Research on tuberculosis and other mycobacterial infections. Resistance to anti-bacillary drugs in Cuba

* Research to confirm that Cuba is free of poliomyelitis. Studies on the circulation of virulent polio virus. National antibody surveys in connection with vaccination

* Studies on HIV infection and comprehensive care for HIV/AIDS patients

* Methods of integral control of vectors of medical significance. Programme for monitoring resistance to insecticides. Biological control of vectors using bacterial and parasitic larvicides and larva-eating fish

* Factors affecting the presence and abundance of river molluscs. Their importance in the epidemiology and control of tropical diseases

* Improvement of the diagnosis and management of acute respiratory infections of viral and bacterial aetiology

* Genetic, biochemical and immunological studies on attenuated strains of Vibrio cholerae.

Pharmaceutical Biological Laboratories (LABIOFAM)

* Production of vaccines against viral diseases in animals: [illegible]; porcine encephalomyocarditis; avian infectious bronchitis; avian encephalomyelitis; Gumboro disease; Newcastle disease; contagious ecthyma; canine distemper and hepatitis

* Production of vaccines against bacterial diseases in animals: erysipelas; bovine brucellosis; bovine bacillary icterohaemoglobinuria; leptospirosis; Neu-S-Suis (Salmonella typhimurium, Salmonella choleraesuis and avian pasteurellosis)

* Development of biological products for vector control: the larvicides "Griseler" (Bacillus sphaericus), "Bactivec" (Bacillus thuringiensis); and the rodenticide "Biorat" (Salmonella enteritidis).

INSTITUTE OF VETERINARY MEDICINE

- Successful completion of the bovine tuberculosis elimination programme

- Vaccination using nationally-produced immunogens to control eastern equine encephalomyelitis, Newcastle disease, symptomatic anthrax and many other diseases which affect animals and in many cases may also affect humans (zoonoses)

- Elimination of bovine brucellosis from more than 90 per cent of livestock, including all the dairy herds

- Efforts to keep the country free of most serious diseases affecting animals

- Development of a vaccine against porcine viral encephalomyocarditis

- Production of biological and other means of diagnosing enzootic diseases in domestic animals.

Finland

Finland has the honour to inform that she has not made any scientific and technological developments relevant to the Convention.

Switzerland

Scientific and Technological Developments
relevant to the BW-Convention

During the last decades biotechnology and genetechnology have revolutionized (and is still doing so) many areas of biological and medical sciences. The possibilities of studying and manipulating genetic information have provided a huge amount of knowledge on basic principles of life. Numerous peaceful applications of this knowledge are employed today worldwide in solving problems of global interest; such as public health and environmental problems.

But soon after the beginning of this biological revolution, it was recognized that these technologies might also have a dark side: their military misuse to create novel Biological Warfare agents.

The possibilities offered by genetic engineering and new biotechnological production capabilities, especially by recombinant DNA (rDNA) and monoclonal antibody (mAb) techniques, to manipulate micro-organisms and parts of them seemed to be almost unlimited. At the beginning of the 1970s BW agents were considered as weapons of little military value, mainly due to their uncontrollability, their unpredictable efficacy and their difficult methods of large-scale production. Although there has been (and still is) a lot of speculation scientists recognized rDNA techniques as ideal means to eliminate these undesirable characteristics of BW agents. As a result many countries have intensified their defensive BW research programmes.

These developments have also been considered with concern and extensively discussed by the States Parties to the BW Convention on the occasion of the Review Conferences. There is a general understanding that the scientific and technological developments relevant to the Convention are covered by the scope of article I of the BW Convention.

Since the last Review Conference in 1991 further advances in science have refined and improved the methods of bio- and genetechnology and some new techniques have been added to the molecular biologist's toolkit, many of them aimed to ease in some way the creation of altered organisms or cells. As offensive BW research is looking for means to alter the characteristics of pathogens in a way that they meet the requirements for militarily suitable biological and toxin weapons, a variety of these techniques have an impact on the development of such warfare agents.

Besides the scientific and technological developments already outlined, as the third Review Conference approaches, progress was made in the bio-production capability such as computer-aided bioreactors with continuous flow systems and integrated biosensors. Today, numerous genetic factors of interest in biological warfare like toxins, resistance and virulence factors, biochemicals and others can be quite easily characterized, isolated and transferred to other host organisms by means of improved recombinant DNA techniques. The polymerase chain reaction (PCR) has developed to one of the most powerful tools for identification, characterization and analysis of genetic material. In the last few years, PCR has been varied and improved continually. Nowadays there is a huge amount of different applications of this method. With the simple and rapid <u>in vitro</u> amplification of genetic material PCR not only offers the possibility of identifying small amounts of specific DNA sequences but is also suited to study DNA from highly pathogenic organisms. By using primers differing at one or several positions from the target sequence it is even possible to modify a known DNA sequence at specific sites. Traditional cloning of DNA relying on technically difficult processes of <u>in vivo</u> replication of the target sequence in a host organism was previously restricted to experienced molecular biologists. The simplification of DNA sequence isolation of PCR has made gene analysis accessible to scientists with less experience in molecular biology.

Biological toxins are increasingly used in medical research and therapy. Consequently there is worldwide an increasing demand for such highly toxic substances. Biotechnology and genetic engineering provide methods for rapid and cheap mass-production of highly potent toxic proteins. The possibility of producing large amounts of toxin within a few weeks resolves the problems arising from stockpiling biological material with relatively low stability, making toxins well-suited as warfare agents.

Besides a possible misuse for developing new biological warfare agents genetic engineering and derivative technologies are extensively employed in research on protective measures against such weapons.

Recombinant DNA techniques are used to develop new and safer vaccines. During the last few years viral vector-based vaccines, split vaccines and synthetic peptide vaccines against numerous diseases have been developed. These may improve the protection not only of a target population against a possible biological attack, but of the aggressors' own troops as well. As a "side-effect" of modern vaccine research, methods may be further developed to insert toxin genes into the genetic material of various viruses.

Highly specific monoclonal antibody fragments can be produced rapidly and in high amounts by new methods (Lerner & Benkovic). These could be used for protection against biological and toxin agents by passive immunization. Monoclonal antibodies coupled with micro-electronics could serve as useful tools in rapid detection and identification of numerous pathogens. Such diagnostic tests based on biosensors are not only a prerequisite for an efficient defence against a biological attack, but they may also be valuable instruments for a future verification of the BW Convention.

These scientific and technological developments are still covered by the scope of article I of the Convention. However, genetically manipulated micro-organisms, unknown viruses, biological toxins, bioregulators and biochemicals still have an increased importance as potential biological warfare agents. Such weapons can be covertly produced and stored on small sites.

The methods of bio- and genetechnology became widespread and are therefore more and more accessible to less experienced scientists, which enhances the danger of proliferation of BW technologies. This danger can be met, among other things, by strengthening the Convention with an effective verification regime.

United Kingdom of Great Britain and Northern Ireland

NEW SCIENTIFIC AND TECHNOLOGICAL DEVELOPMENTS RELEVANT TO THE BIOLOGICAL AND TOXIN WEAPONS CONVENTION

1. Introduction

1.1 Article XII of the Convention on the Proliferation of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction (BTWC) provided for a conference of States parties to

be held to review the operation of the Convention within five years of entry into force. This review was mandated, inter alia to "take into account any new scientific and technological developments relevant to the Convention".

1.2 To assist the work of the First Review Conference in 1980, the Co-depositaries presented a paper on new scientific and technological developments relevant to the BTWC. They prepared separate national papers for the Second Review Conference in 1986. For the Third Review Conference in 1991, papers were prepared by the Depositaries and a number of other States parties. Papers of this type have again been requested by the Preparatory Committee for the Fourth Review Conference due to be held in 1996.

1.3 The previous United Kingdom papers considered advances and changes in science, medicine and industrial applications in the interim since the previous Review Conference, and discussed the implications for scope of the Convention and for the balance between the potential for misuse of science and technology and the potential for improvement in defensive measures. This approach will again be adopted in the present paper, but additionally consideration will be given to any implications for the ongoing work of the Ad Hoc Group mandated with drafting a legally binding instrument to strengthen the Convention.

2. General developments relevant to the BTWC

2.1 There has been a steady increase in the number and scope of biotechnology applications, with better integration of knowledge and techniques contributed from an increasingly wide array of fields such as microbiology, biochemistry, molecular biology, cell biology, immunology, protein engineering, classical breeding, bioprocess technology, and the traditional biotechnology applications of micro-organisms in producing beer, wine, cheese, and other foods. Many commercial and academic projects involve new relationships between the microbiologist, the molecular biologist, and the chemical engineer, and ad hoc relationships are being reinforced by educational programmes such as those under the European BIOTOL (Biotechnology by Open Learning) programme.

2.2 The study of genetics and the application of genetic modification technologies in fields relevant to the BTWC has increased markedly in the five years since the Third Review Conference. Such technologies are being ever more applied in research in the medical and pharmaceutical sector, but there has also been a widening of scope with achievements in the agriculture and food sectors. Some of these advances are reported below. There has been a dramatic increase in knowledge of the genetic basis of pathogenicity of some bacteria and viruses, with genetic sequences available on the internet; and there is an increasingly global dissemination of research techniques that allow cloning of genes including genes for toxin and toxin subunits into a variety of host types - the very large number of successful clonings into micro-organisms is now being augmented with reports of the creation of transgenic animals and higher plants. Should a proliferator adopt a high-tech route for weapons development, the potential range of options has thus increased markedly in this time period.

2.3 Attention to biosafety issues had continued, with increasing development of national regulations covering contained use of micro-organisms and toxic chemicals, and contained use or deliberate release of genetically modified micro-organisms (and other organisms). There has also been increased international cooperation on safety in biotechnology: for example he commitments of Governments under Agenda 21 on sharing experience, capacity-building and international agreement on principles for safety. This commitment has already seen the development of technical guidelines by the United Nations Environmental Programme in 1995.

2.4 Micro-organisms may well be used routinely in the important disarmament context of the Chemical Weapons Convention. Microbial bioremediation is now established as one of the candidate methods for destroying bulk stocks of mustard in national programmes for the destruction of chemical weapons stockpiles. Similar methods may also be applicable to soil contaminated with a range of CW agents and other toxic chemicals.

2.5 Awareness of responsibilities and issues occurring under the aegis of the BTWC, including the work of the Ad Hoc Group in Geneva, has been improved by the information explosion allowed by the internet, which links around 2 million computer systems around the world. The question of whether the internet could have a role in measures included in a future instrument to the Convention needs further consideration.

3. Detection technologies

3.1 R & D into generic and specific detection techniques has continued to address microbiology applications in clinical, food and environmental settings and in biological defence. The possibilities for aerosol characterization have advanced due to the development of multi-parameter characterization systems based on features such as particle size, shape, fluorescence and polarization of light, and a range of commercial equipment is now available. Detection of adenosine triphosphate is used as a generic test for the presence of live organisms in a commercial monitoring device. Flow cytometers have been miniaturized to the point where they are portable; depending on the indicator principles applied, such devices have the potential for generic or specific detection of bacteria. Following the lead of pregnancy test kits developed as hand-held devices and dipsticks, a number of microbial detection techniques based on antibody and gene probe principles have been developed into this type of format.

3.2 Research and development of sampling devices and rapid detection and identification systems for potential BW (biological weapons) agents in a biological defence context is continuing. A range of generic and specific approaches are being considered. Indicator techniques for antibody-antigen reactions include potentiometry, chemiluminescence and fluorescence; other detection principles include laser light scattering in flow cytomerty, and pyrolysis and electrospray mass spectrometry. The latter has recently been used by CBD Porton Down to identify cricket paralysis virus. Information systems to combine data from multiple detector arrays are also being worked on. Biological agent sampling and detection systems were deployed by Allied forces in the 1991 Gulf War.

3.3 The high specificity and sensitivity of specific microbial diagnostic techniques based on PCR (polymerase chain reaction) principles, with the potential to detect as few as 10 micro-organisms, has led to a great increase in the number of research applications, but the potential of PCR technologies in routine diagnosis for example in the clinical laboratory has still to be realized. High cost of reagents, the need for specialized staff for manual techniques and the lack of availability and high cost of automated techniques continue to be factors. However, methods are under development for mycobacteria, HIV, Hepatitis viruses, and Salmonella in foodstuffs. Other nucleic acid amplification methods are also now appearing. Environmental pressures are leading to the development of indicator strategies that do not require the use of radio-labels, but use either absorption or fluorescent dyes. Such approaches are being incorporated increasingly into dipstick formats.

3.4 The above technologies may be worthy of consideration by the Ad Hoc Group in the context of its work on measures that could be included in a future instrument to the Convention.

4. Genetics developments

4.1 It is predicted that the human genome will be sequenced by the year 2005. The information is expected to lead to radical new treatments for a broad range of human disease, but it may also provide information about biochemical pathways that are susceptible to peptides and to other compounds, which could thus be exploited as a hostile act to damage human health. It cannot be ruled out that information from such genetic research could be considered for the design of weapons targeted against specific ethnic or racial groups. However, it is far from clear that the development of such weapons could ever be anything more than a theoretical possibility. Genetic weapons of this type would be a clear contravention of Article 1 of the BTWC.

4.2 Improvement in the speed of automated techniques for high volume DNA sequencing has led to remarkable achievements over the last five years. Many more laboratories have these sequencing capabilities, and are considering total genome sequencing. Genomes from some of the medically important viruses have been sequenced, and three complete bacterial genomes have been published including the pathogen Haemophilus influenzae. Another half dozen or so medically significant Staphylococcus and Streptococcus species are believed to have been sequenced by industry, but these sequences are unlikely to be placed in the public domain. A further 30 or so genomes will probably be sequenced in the near future. Information from genome sequencing has provided insights into the organization of the genome, and this can be expected to lead to a fuller understanding of how it functions. This should allow identification of housekeeping genes and virulence factors, possibly leading to the discovery of new toxins or immunomodulators.

4.3 The polymerase chain reaction has changed molecular biology radically. It has proved invaluable for the cloning and manipulation of natural DNA sequences, and also makes it possible to create new DNA fragments specified by the scientist. This can be done by using PCR in combination with chemical methods to make longer fragments of DNA, which can then be amplified using PCR to make ample amounts for cloning into possible vectors. Unfortunately, this

means that a proliferator now has the potential to clone, say, toxin genes without actually needing the naturally occurring organism as a genetic source. Many more microbes are now amenable to genetic modification either in terms of cloning DNA from them or using them as vectors for carrying and expressing foreign genes.

4.4 Though much of the achievement in the application of genetic modification technologies comes from the improvement of well established methods, new methods continue to appear. An example that may see widespread use within a few years is phage display, in which libraries of filamentous phage expressing random peptide fragments are screened to select molecules with particular binding characteristics. Amplification of the appropriate phage then allows the recovery of the peptide of interest. Screening in vivo has recently been used to find phage that home to specific organs; this approach has the potential for use to identify specific target peptides in tumours or other diseased tissues, allowing the targeted delivery of drugs or of viral vectors in gene therapy.

4.5 Most microbial toxins have been cloned into micro-organisms. Many non-microbial toxins in their natural state are impracticable to produce on any significant scale. However, such are the advances in cloning success rates that at least the protein toxins can now, in principle, be produced on a large scale by growth of a recombinant microbial or eukaryotic cell host using well-established fermentation technology. The genetic sequences of such toxins have been deposited in the public domain molecular biology databases which are growing exponentially. Although in principle there may be an increasing potential for BW proliferators to mix genetic sequences in order to develop hybrid toxin BW agents, the fact that more than 240 bacterial toxins are already known - to say nothing of the snake, spider, and fungal toxins - means that a proliferator would not need to go to such lengths. Application of genetic modification technologies to naturally occurring bacterial toxins can increase production yields in the primary culture medium by 50 fold or more relative to wild type strains, and expression in a different organism can greatly reduce purification problems.

4.6 Much more is now know about the structure-function relationship of various toxin groups. The combination of electrochemical and other biophysical techniques with molecular biology approaches is expected to lead to the resolution of the molecular mechanisms of cell penetration by protein toxins. There is now a substantial amount of research on hybrids of toxins and toxin subunits with antibodies and viruses, often with the long-term objective of specifically destroying diseased cells such as cancer cells. In this type of therapy an antibody-toxin complex would be injected into the blood stream; the antibodies attach to receptors on the target cells which are subsequently eliminated by the action of the toxin, while healthy cells elsewhere in the body are unaffected.

4.7 There have been increasing numbers of reports of the creation of transgenic animals and plants, and for example the genes for ricin have been inserted into tobacco plants. Such achievements may complicate the focusing of declaration modalities under a future instrument designed to strengthen the Convention.

4.8 The applications of genetic modification in research projects with long-term objectives for disease therapy is rising exponentially. In this field there is a general trend away from work with bacteria, principally disabled strains of Escherichia coli, towards eukaryotic viral vectors. As information on human genetics becomes available for example from the Human Genome Project, viruses are being increasingly used as delivery systems to transfer human genes either into mammalian cells in tissue culture or into whole animals as part of gene therapy research. Gene therapy, still in its infancy, involves the insertion of genes into individuals suffering from genetic diseases. One early success story is that the isolation of the genes associated with cystic fibrosis has allowed treatment by delivering wild type DNA through the lungs: this causes reversion to wild type of the alveolar cells, which lasts until a new layer of mutant cells grow up from the underlying germ cells.

4.9 Viral vectors are an attractive starting point for gene therapy because viruses have evolved efficient mechanisms to introduce and express their nucleic acid in recipient cells. The snag is that the host cells have evolved sophisticated mechanisms to rid themselves of viruses. The challenge for gene therapy research has therefore been to achieve efficient and extended expression of the foreign gene while evading the host defences. Two of the more commonly studied delivery systems are retroviruses and adenoviruses, with cancer applications predominating. Genetic modification technologies could be applied to retroviruses, change their tissue tropism and thus overcome the disadvantage that they cannot invade non-dividing cells. In a different context, an example of a successful change in tissue tropism has been publishd for chimeric TBE and Dengue Type 4 viruses.

4.10 The sequencing of complete viral genomes has provided new insights into the molecular basis of virus virulence. The complete genome sequence has been published for four strains of VEE virus and for the attenuated vaccine derivative of one of these strains (TC83); only two mutations were found to be required to convert an infectious cDNA clone of TC83 to full virulence for adult mice. Chimeric viruses have been produced by genetic recombination in order to allow growth of viruses in easily grown cell lines, for production of vaccines or as a strategy for therapeutic drug production. However, chimeric viruses produced by a BW proliferator could circumvent specific detection systems and the immunity acquired by vaccination. A range of viruses that are infectious for man, including vaccinia, adenovirus and VEE virus, have been used as expression vectors to express foreign genes. In principle, a BW proliferator could use such virus systems as vectors for bacterial or other toxins.

5. Vaccines

5.1 The global eradication of smallpox has been sustained, and is thus a testament to the potential of vaccination in disease control. The global eradication of polio is already well advanced and the disease is now rare in the Americas and Western Europe. Changes in economic conditions have led to an increase in vaccine development initiatives in some countries, in many cases based on novel technologies. Practical and ethical problems associated with the human use of vaccines based on recombinant organisms have to date tempered the promised advances in genetically attenuated live vaccines.

However, some recombinant human vaccines have been in use for several years and an example is hepatitis B cloned into yeast - which illustrates the potential production advantage of a recombinant vaccine that is based on a non-pathogenic host and that can thus be produced and handled under low levels of containment. Genetically modified animal vaccines have also started to appear. An animal vaccine that uses capripox virus as a vector to carry genes for Rinderpest virus antigens. This vaccine, which has the advantage of simultaneously protecting cattle against lumpy skin disease and Rinderpest, is currently undergoing trials in Kenya in closed conditions. Another recombinant example is a rabies vaccine for doctoring bait intended to be distributed in the wild as a means of immunizing foxes.

5.2 Recombinant vaccine designs, however, often turn out to be less protective than classical vaccines. There has therefore been increased interest in identifying improved adjuvants, though many candidates trialled to date have proved too reactogenic for general use. Also, increasing importance is now attached to the significance of cellular immunity in vaccine action, particularly in virus infections, as a better means to mimic the real infection. There has been considerable research in the use of new adjuvants, delivery vehicles and immuno-modulators to direct the immune response towards TH1 responses, and one approach is to use toxin-derived molecules. Subunit combination vaccines have been successfully developed, for example based on Diphtheria/Tetanus/acellular Pertussis/Haemophilus influenza type B/hepatitis B, but attempts to increase the number of components tend to be counterproductive because they lead to antigenic overload. A number of vaccines have been formulated for intranasal, oral and slow release delivery. Mucosal vaccination has received increasing consideration, and it is clear that induction of immunity at mucosal surfaces can lead to a disseminated systemic response. These new administration route strategies offer not only logistic advantages for routine use but also the prospect of increased protection against infection by the respiratory route - a factor which could have relevance for biological defence programmes.

5.3 In spite of the efforts being put into vaccine development, effective vaccines are still not available for some important diseases. Thus, current influenza vaccines are still only about 80 per cent effective and duration of immunity is one to two years, shorter than after a natural infection.

5.4 Where the level of disease burden has reduced in a country, it is now recognized that the need for vaccine boosting increases. There has been an increase in the range of serological tests for antibody titres, and in the tests available for functional activity such as bactericidal or virus neutralizing activity, and the trend towards increased emphasis on quality assurance has seen global studies aimed at validating new test methods and standardizing existing methods.

5.5 A major new vaccination prospect that has become firmly established since the Third Review Conference is the use of DNA vaccines. Naked DNA is sued as an inoculum that is taken up by cells where the genes can be expressed for up to two years. The first applications will probably be in vaccines for viral diseases such as influenza and hepatitis A-C, but there is also a massive potential for cancer vaccines. No adjuvants are required, and delivery by injection, transdermal or oral routes is possible. As with other

novel technologies applied to vaccine designs, safety issues will remain high on the agenda. Ethical questions remain about use in healthy humans, and the understandable need to demonstrate the absence of any risk from indirect effects means that progress to clinical trials cannot be hurried. But DNA vaccines at the research stage show promising results in animals, and vaccine development should be much quicker and cheaper than for any genetic modification approach in a microbial vector because one avoids the manufacturing complication of having to express a protein and then carry out downstream processing.

5.6 Because of the trend towards recombinant vaccines and the attention being paid to vaccine efficacy, combined with the strides that have been made in understanding and controlling fermentation processes, the fermanter scale needed for routine vaccine production is reducing fast. Commercially viable operations based on fermanters in the tens of litres range rather than hundreds or thousands of litres will become the norm. Where recombinant vaccines involving safe hosts are produced, low levels of containment will be acceptable. These trends in vaccine manufacture have implications for declaration and compliance monitoring arrangements under the BTWC.

5.7 Some countries have a substantial emphasis on vaccine development, trialling and production in their biological defence programmes.

6. Trends in infectious diseases and their treatment

6.1 There have been important successes in international efforts to alleviate disease burdens. Largely through the activities of international agencies such as the WHO, smallpox has been eradicated. The WHO eradication programme for polio virus by the year 2000 is on target and a programme for measels has started though there is evidence that vaccination may be stimulating antigenic drift among the pathogenic measles viruses in circulation. Great strides have been made in controlling dracunculiasis, leprosy, Chagas disease, neonatal tetanus and onchocerciasis (river blindness). Nevertheless, there is increasing concern about the potential for international spread of disease and about incidents involving emerging and re-emerging diseases and in 1995 the Member States of the World Health Organization called upon the Organization to strengthen and coordinate global surveillance and control of communicable diseases. In response to this resolution, the WHO in October 1995 established the Division of Emerging and other Communicable Diseases, in order to bring together WHO's existing surveillance activities and to promote the development of national and international infrastructure and resources to recognize, monitor and respond to communicable diseases and emerging health problems.

6.2 In spite of the attention placed on hygiene of foodstuffs and water supplies, significant disease outbreaks continue to be reported in developed countries. Particularly problematic are *Escherichia coli* 0157 in water and food and *Cryptosporidium* in water supplies. Antibiotic resistance continues to be a major threat to disease control, and the impact of multiply resistant *Mycobacterium tuberculosis* has only been mitigated by the fact that it appears not to spread very easily.

6.3 The uncontrolled use of antibiotics is implicated in the emergence of the many resistant strains of bacteria which are causing serious problems in hospitals, for example methicillin-resistant Staphylococus aureus. The problem is compounded by the poor discovery record for new antibiotics. The situation may however improve as pharmaceutical companies focus their research on the newly elaborated genome sequences of bacterial pathogens in order to seek new targets for antibacterial drugs effective against multiply resistant strains. The introduction of combinatorial chemistry has greatly increased the number and complexity of compounds that can be generated and screened for therapeutic activity, and it seems that this strategy will replace the random screening of secondary metabolites. Information gained from genome sequencing and from advances in protein engineering and structure analysis may allow rational drug design, but these technologies also raise the spectre of rational design of novel toxic compounds.

6.4 Several newly recognized human viral diseases have emerged within the last few years. Examples that cause fatal human respiratory disease include hantavirus pulmonary syndrome in the United States of America and equine morbillivirus in Australia. Other viruses that have not been detected in humans for several years have re-emerged, for example Ebola and epizootic Venezuelan equine encephalitis. There is evidence that increased contact of humans with vectors has led to new human viral diseases and the rapid spread of established ones, for example the incidence of Dengue associated with the increase in the mosquito vector in Central and South America. Other infectious diseases that are causing concern include Rift Valley Fever, Yellow Fever, Rocio, Hantaan, Sabia, Lassa Fever and plague. In spite of the considerable effort to produce anti-viral drugs effective against important disease agents such as HIV and Herpes virus, clinical trials have been disappointing because these drugs appear to exert a selection pressure for resistant genotypes of the virus. The growing application of molecular biology to anti-viral design is expected to lead to designer targeting of molecules which are essential to the survival of the virus and which become non-functional if they mutate.

6.5 The recent emergence of the prion based disease spongiform encephalopathy in cattle (BSE) and its possible transmission to man to cause a new form of Creutzfeld-Jacob disease (CJD) indicates the potential socio-economic consequences of disease even when it has a very low risk of transmission to humans (only a dozen such CJD cases have been reported to date in the United Kingdom). Infectivity experiments with animals indicate that transmission of this prion requires direct brain inoculation. Although such prion agents are extremely resistant to inactivation and are probably infectious by the aerosol route, they are poor candidates for BW use because of their very low transmission potential and the very long incubation period between exposure and disease. Prions, a malfolded version of a normal cellular protein, clearly fall within the scope of Article 1 of the Convention.

6.6 Research has continued into new compounds active against plant pathogens, but there are still no anti-virus compounds and no good plant bactericides except for antibiotics (which are not approved for crop application in the United Kingdom and are relatively expensive for such use). There is still a lack of fungicides effective against the important wilt

pathogens in the genera Fusarium and Verticillium. Genetic modification of plants to express viral coat proteins and thus confer some protection against infection by the virus or viruses concerned is now relatively commonplace in research but such plants have not yet been released for commercial use in the United Kingdom. Monoclonal antibodies for use in disease detection have been produced with wide and narrow specificity and there are now serological methods for the detection of haptens and plant pesticide residues.

7. Industrial microbiology

7.1 There is a trend towards choosing "workhorse" host organisms for industrial genetic modification applications, as the standard organisms into which to insert genetic material. An example is the established safe organism Escherichia coli K12. This approach offers the advantage of well understood organisms with fast growth and high productivity, that can be used under standardized fermentation techniques. On-line biosensors will lead to better control of processes. All these developments are expected to contribute to higher productivity, thus allowing the use of modestly sized equipment - which has implications for the focus of future declaration and on-site measures under the BTWC.

7.2 Micro-organisms are being used in biotransformation processes to carry out stereo specific chemical transformations on molecules which are the active form in a variety of pharmaceuticals and agrochemicals.

7.3 The application of biotechnology in the manufacture of a greater range of pharmaceutical and high added value products has been accompanied by an increased attention to quality standards and environmental control in some industrial sectors. This has resulted in more widespread use of containment measures designed to protect the product but often having the dual effect of protecting personnel from the process. The growing use of high quality chemical engineering in fermentation processes in all geographical regions of the world also increases the opportunities for misuse of this technology for the production of pathogens or toxins as BW.

7.4 An example of the versatile tools that have been developed for the expression of a variety of heterologous proteins in industry is the baculovirus-insect cell system. Stable baculovirus vectors can be constructed rapidly and with a minimum of viral manipulation. In the last decade, developments in bioreactor design and the use of cell-protective additives have helped to mitigate the sensitivity of the insect cell lines to agitation and sparging and their relatively high oxygen demand. New, serum free media formulations have allowed cost reductions and easier purification of recombinant proteins, which for baculoviruses have included ricin and mammalian derived prion proteins.

7.5 High level protein expression has also been achieved using Semliki Forest virus grown in a range of mammalian cells. The heterologous protein product can constitute up to 25 per cent of total cell protein, and, starting with the target gene, an infectious virus can be obtained in only one to two weeks. Using a viral mutant which is non-infectious allows scale up with good safety control.

7.6 Practical studies to monitor releases in industrial operations have exploited the high sensitivity of PCR techniques. In combination with computational fluid dynamics to determine the trajectory of release, spatial distribution and concentration of cells within a bioprocessing area, PCR has been shown to detect specific genetic material even in the presence of related organisms. The development of biosensors as "artificial noses" that could be used in exit gas streams is becoming feasible.

8. MICROBIAL CONTROL OF PESTS

8.1 Bacillus thuringiensis (Bt) continues to be the most important agent being used to control insect pests. From fundamental studies of the mode of action of these bacteria and the use of advanced genetic modification techniques, Bt toxin genes have been transferred to create transgenic plants. This enables the plant to protect itself from pests by producing an insect-specific Bt toxin. The first major releases of commercial crops based on this principle have occurred, heralding what could turn out to be a major new era in environmentally benign farming avoiding the use of chemicals. The bacterium Bacillus sphaericus, pathogenic for some mosquito species, is another promising biological alternative for pest control.

8.2 The use of viruses to control insect pests has been advanced with the appearance of new commercial pesticides based on the nuclear polyhedrosis virus to control pest species resistant to chemicals. Genetic modification is also being used to improve the performance of these pathogens: new, faster acting strains of virus have been created by incorporating into the genome specific insect toxins derived from other animals such as scorpions. The creation of transgenic plants incorporating genes of insect viruses is also a promising new line of development in crop protection.

8.3 Fungal agents such as Metarhizium spp, and Beauvaria bassiana are being actively developed as biological control agents against important pests such as locusts in Africa and aphids on field crops.

8.4 Advances have been made in developing specific microbes to control noxious weeds such as Rotbellia in South East Asia. The study of the molecular biology of crop diseases like bacterial rots, bacterial wilts, fungal blights and plant viruses has provided the basis to develop the specific diagnostic tests needed by plant propagators to produce disease-free planting material for farmers.

8.5 Of these developments, the direct attack of insects may seem unlikely as a means of biological warfare. However, technologies able to insert genes for viruses or toxins into animals or higher plants could increase the production options for a proliferator. Even entirely legitimate uses of such transgenic plants may create complication for future declaration and inspection measures under the BTWC.

9. CONCLUSIONS

9.1 The three previous papers on new scientific and technological developments prepared by the United kingdom for the earlier review conferences concluded that the rapid pace of scientific and technological developments in

areas relevant to the BTWC demonstrated that implementation of its provisions had not hindered activities for peaceful purposes. The present review of developments up to 1996 indicates that there continues to be no impediment.

9.2 The United Kingdom continues to hold the view that the BTWC fully covers all microbial, other biological agents and toxins, whether naturally occurring or not, including any resulting from the application of genetic modification or other technologies. Technology advances and scientific discoveries over the last five years have not weakened this assessment.

9.3 Each of the earlier papers in this series reported an increased potential for the large-scale production of BW agents, and an increasing technology potential that could be misused to alter candidate-BW agents in order to address shortcomings in their characteristics from a weapons viewpoint or to increase their production potential especially in the case of toxins. Though there has been no quantum technology leap in the intervening five years, the current review indicates that this trend has continued. Increasingly worldwide use of technologies including bioprocessing in the civilian sector have further increased the opportunities for BW proliferation. The markedly increased number and scope of the applications of genetic modification technologies could provide an ever widening range of options for a proliferator choosing a high-tech route for BW development. This could include engineering properties into agents to improve their stability, infectivity, ability to escape the notice of deployed detection and identification systems, or overcome the immunity of target populations.

9.4 There is, however, nothing to indicate that a proliferator having the technical capabilities to adopt a high-tech route is any more likely to do so than formerly, and use of these technologies in an illegal programme will tend to be accompanied by an increased risk of failure and of detection particularly under the operation of a future instrument to the Convention.

9.5 The potential for improving defensive measures against possible BW attack, both in respect of detection and identification techniques and the procurement of improved vaccines, has further improved since the previous Review Conference. The advances in specific identification technologies reviewed in this paper may also be relevant for the measures being considered by the Ad Hoc Group.

9.6 Technology developments such as the reduction in scale and containment levels of vaccine production resulting from the use of more efficient, and recombinant vaccines, and the increasing use of transgenic species including crops, need to be considered in the work of the Ad Hoc Group.

United States of America

TECHNOLOGICAL DEVELOPMENTS OF RELEVANCE TO THE BIOLOGICAL AND TOXIN WEAPONS CONVENTION

1.0 Introduction

1.1 In preparation for the 1996 Review Conference on the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological

(Biological) and Toxin Weapons and on their Destruction (BWC), the Preparatory Committee requested the depositary nations to prepare national papers on new scientific and technological developments relevant to the Convention.

1.2 Since the last Review Conference in 1991, there have been a number of developments and advances in the field of biotechnology, some of which can contribute to improvements in the compliance picture for the BWC. The major breakthroughs have occurred in the fields of analytical developments and vaccinology, both of which have application in the field of medicine and public health as well as industry and agriculture. Of special interest to the BWC are applications in detection and identification which can enhance compliance and production applications which provide further concerns regarding compliance. The number of countries which are developing a biotechnology capability continues to grow as the applications continue to expand into commercial sectors and the resulting industry has expanded in both scope and products developed and marketed. All of these trends continue to have practical significance for the BWC and the problems of compliance with the BWC.

1.3 Our review of technological developments must continue to encompass a broad overview of a wide variety of fields to include basic research through manufacturing in such areas as: biotechnology, molecular biology, medicine, microbiology, biochemical engineering, and pharmaceutical and vaccine production. This activity could include inputs from the academic community, industry, government agencies and departments, trade organizations and similar knowledgeable personnel. The significant advances in assay technology represent positive developments since these can contribute to rapid detection and identification, a critical area in BWC compliance. At the same time the advances made in new, simpler and more rapid production and manufacturing technology remain a BWC concern as well since these developments can contribute to further proliferation. Our concerns expressed in 1991 remain, that while promising great benefits to mankind the advances in technology could be used to produce new substances or modify old ones and lead to a new and significant toxin, biological or biochemical weapons threat and we all must remain aware and cognizant of this potential.

2.0 Advances in industrial application of biotechnology

There are a number of industrial areas where advances in production and assay technology as well as new product technology have relevance to the Convention. These will be detailed in the following sections.

2.1 Modified micro-organisms: Biotechnology allows the development of micro-organisms and subsequent products with new and unique characteristics, many to meet specific purposes. Examples of some of these engineered products are monoclonal antibodies which contain a variety of toxins for therapeutic purposes in treating some leukemias and cancers. Others in various stages of development involve Pseudomonas exotoxin and diphtheria conjugates for cancer and AIDS treatments. In the area of plant pathogens, these same developments are being pursued to produce environmentally safer and more effective pesticides. All of these developments can provide significant benefits to the public health and welfare of society, however, in examining these same developments from the point of view of the BWC, we cannot ignore the potential

misuse of biotechnology to produce new biological agents or improve certain characteristics in those agents already recognized as potential biological agents. Transferring certain genetic characteristics into naturally occurring organisms can potentially create organisms of greater virulence, antibiotic resistance and environmental stability. Changing the microbes genetically could alter their immunogenicity, thereby rendering vaccines and serodiagnostic techniques useless. Otherwise harmless micro-organisms could be altered to produce toxemia or disease, although the host animal would continue to recognize these micro-organisms as innocuous and therefore not defend against them.

2.1.1 Bioengineering of micro-organisms has other implications for the BWC as well. Bacteria and yeasts, genetically altered to produce products, are miniature factories by virtue of their ability to reproduce rapidly. Examples are the production of a wide variety of products by the insertion of genes into tobacco mosaic virus and product production by infection of the tobacco plants with the virus and subsequent product extraction and purification from the plant. Large quantities of compounds, previously available only in minute amounts from natural sources, are now available quickly and easily. Such production methods are now becoming commonplace in industry.

2.1.2 It is now possible to identify genes which have desirable properties, isolate them and transfer them between host organisms. A nearly infinite variety of biological compounds designed for specific uses and given specific characteristics is possible. For example, this should make specific vaccine developments much easier to accomplish. Given the technical progress in this area, future developments should be of concern to the BWC Review Conference. Within the next decade, the potential for misuse of ongoing developments in biotechnology could be most pronounced in the following areas:

2.1.3 Microbial pathogens could be genetically engineered to maximize infectivity and pathogenicity. Likewise they could be modified to increase or decrease their environmental stability and persistency.

2.1.4 Toxins. Naturally occurring protein toxins normally available only from natural sources in small quantities could be made in host organisms by modifying their DNA. Plant and/or fungal toxins could be mass produced. If used as an agent, the origin of these toxins could be difficult to pinpoint, given that they are already present in the environment, albeit at low concentrations. Improvements in biotechnology since the previous Review Conference lead us to believe that production of potent toxins, which until now were available only in minute quantities, and only upon isolation from immense amounts of natural biological materials, can now be produced in kilogram quantities which could be militarily significant.

2.1.5 Peptides. Peptides have been called "the antibiotics of the year 2000" because these biological materials may represent a new class of miracle drugs. Peptides are precursors of proteins made up of amino acids. They are interesting molecules for many reasons. They are active at very low concentrations (one part per billion or trillion) which makes their detection very difficult. They can be successfully modified as agonists (more active products) or antagonists (having a contrary activity). For example,

modification of LHRH, a fertility hormone, by substituting a single amino acid has yielded a product 50 times more potent. Another modification of this same peptide yields a product useful in the treatment of prostate cancer.

2.1.6 Their range of activity covers the entire living system, from mental processes (e.g. endorphins) to many aspects of health such as control of mood, consciousness, temperature control, sleep or emotions, exerting regulatory effects on the body. Even a small imbalance in these natural substances could have serious consequences, inducing fear, fatigue, depression or incapacitation. These substances would be extremely difficult to detect but could cause serious consequences or even death if used improperly.

2.1.7 The predictable modification of peptide and protein structure and function (i.e. protein engineering) is in its infancy. Computer-aided molecular design will rapidly develop, enabling molecules to be manipulated for varying degrees of physiological activity, specificity and stability. Technologies permitting the direct chemical synthesis of peptides and proteins in large yields will, in the more distant future, augment or replace microbial production of these molecules.

2.2 Advances in production. As mentioned earlier, once a suitable recombinant organism has been engineered, exploiting it becomes a matter of using established procedures. Biological production technology has proceeded to the point where large quantities of biological products can be produced quickly in small facilities. An example is the production of bovine growth hormone, a compound developed to increase milk production. Two genetically altered pesticides are about to be approved for commercial use which represent a "second generation" of biopesticides which will not degrade quickly in the environment due to a microencapsulation process. Long-term refrigerated storage, in some cases will not be needed because large quantities can be produced very quickly starting from a minute seed stock. Several relevant technological considerations regarding biological production are discussed below.

2.2.1 Mammalian cell culture. Recent advances in mammalian cell culture make possible the growth of mammalian cells on the surface of minute beads, rather than on the inner surface of glass roller bottles. These cell culture systems provide the ideal environment for the growth of viruses. The new technique greatly simplifies virus production and allows large-scale yields in facilities of modest size. A further advantage of these cell culture systems is the ease of purification and concentration of the virus produced since it is relatively clean and free of impurities from the production process in contrast to earlier fertile egg production systems. As another example of advances in this field, the amount of tissue culture media needed to produce antibodies has been reduced a hundredfold by the use of encapsulated hybridomas. Such developments are eroding the distinction between production facilities and small laboratories.

2.2.2 Continuous flow fermentors. The introduction of computer controlled, continuous flow fermentors has dramatically increased productivity. Most likely the size of fermentors operating by batch process can be reduced a thousandfold by conversion to a continuous flow process.

2.2.3 Hollow-technology fibre. Hollow fibre technology provides an example of the industrial production potential of the new technologies. This technology permits a far greater concentration of cells with a markedly increased rate of recovery in a shorter time than previously obtained using roller bottles. This equipment occupies less than one twentieth the volume of the previous technology. Concentration and purification of a wide variety of protein substances can now be accomplished in large-scale liquid chromatography columns, yielding pure, highly fractionated proteins. In the isolation of such cellular biomaterials as pyrogens, a similar transformation has taken place. Separation and reconstitution of the product can now be accomplished in about an hour using new compact ultrafiltration methods, whereas older methods took as much as four days.

2.2.4 Safety and environmental standards. Pharmaceutical plants around the world increasingly have incorporated safety and environmental release provisions akin to those which were once unique to BW production facilities for product purity, worker safety and environmental and community protection, making it increasingly difficult to distinguish between permitted and prohibited activities.

2.2.5 Improvements in equipment, speed of production and quality of product are a common occurrence in the history of the commercial development of any new technology. Many oil utilizing micro-organisms produce a surface active compound which can emulsify oil in water and facilitate recovery of the oil. A microbial glycolipid emulsifier has been produced in large quantities and shown to act as a powerful dispersant of oil in water. Unlike chemical surfactants, it is non-toxic and biodegradable. Other strains of micro-organisms have been developed which have the potential for use in biodegradation of chemical nerve agents, mustard, explosives and hazardous wastes such as PCBs. Other commercial developments in the area of agricultural research using biotechnology have occurred in crop research. Cereals, forages, fibre crops and fruits and vegetables that are highly adapted to environmental stresses such as drought, cold, heat and toxic soil minerals are being developed.

2.2.6 In animal research, scientists are developing effective diagnostic tests for diseases of beef and dairy cattle, pigs, sheep, chickens and turkeys, and producing new and more reliable preventative measures against livestock diseases. Other researchers are using the tools of biotechnology to grow bacteria that can break down toxic wastes. Because of the large number of technical innovations in the area of industrial microbiology, it has become more difficult to assess compliance with the BWC since its signature in 1972. Developments intended to increase production, decrease cost and create safer conditions for handling biological materials have blurred former distinctions important for purposes of assessing compliance - for example, between a large production facility and a laboratory. Also, capabilities to break out of the Convention in a very short time have increased.

3.0 Advances in analytical and vaccine technology

Though not without constraints, developing biological and toxin weapons is a much easier task than developing adequate defences against them.

However, the very advancements in biotechnology that have caused increased concern have also put new tools in the hands of those conducting permitted biological research.

3.1 Developments in assay technology: There are a number of developments now available for rapid clinical diagnosis of a variety of potential biological agents.

3.1.1 Enzyme-linked immunosorbent assay (ELISA): This is one of the more widely used assay techniques today. The ELISA assay is based on the interaction of an antibody with its specific antigen and the subsequent detection and assay of this interaction by an enzymatic reaction. ELISA has three steps: first immunoreactants are immobilized on a solid phase to act as capture immunosorbent, second the sample to be captured is added, and third immunochemical reactions are detected by adding specific enzyme conjugated antibodies directed against the sample. ELISA assays are rapid and easy to perform. They are highly sensitive, specific and reproducible. Equipment is relatively cheap. The immunoreagents have a long shelf life when stored frozen, no hazardous radioisotopes are required and large numbers of samples can be assayed simultaneously.

3.1.2 Nucleic acid probe detection: Another effective approach is the use of nucleic acid probe detection which is based on the ability of labeled ribonucleic or deoxyribonucleic acid (RNA or DNA) probes to recognize and bind to complementary target nucleic acid sequences in a sequence-specific manner, through the process of molecular hybridization. The use of nucleic acid hybridization technology has enormous potential in rapid diagnosis which is enhanced through the use of the polymerase chain reaction (PCR) which allows for the amplification of minute quantities of genomic material without extensive purification. The specificity depends upon the stability of the probe-target hybrid formed under the hybridization conditions used such as pH, temperature, salt concentration and probe length, the sensitivity also depends on the hybridization conditions of temperature, time, probe material and the detection system used to detect the labeled probe. Nucleic acid probe detection systems are highly specific and depending upon the probe and the primers used can detect discrete genes. They do require highly trained personnel to perform the assay, contamination of the sample must be prevented, cost may be prohibitive and the test will generally only detect agents with known, unmodified gene sequences.

3.1.3 Biosensors: Biosensors evolved from the combination of two disciplines: modern biotechnology and advanced electronics. Biosensors have advantages over conventional detectors including high sensitivity, capacity for miniaturization and the availability of multiple measurement sites. Rapid, individual analysis can be expedited, often by minimally trained personnel. Three major types of biosensors exist: biocatalytic, immunochemical and receptor based. Biocatalytic are generally based on purified enzymes immobilized on a solid substrate combined with a signal transducer. The immunological biosensors are based on coupling immunological interactions between antibodies and antigens with electrochemical assays which can encompass fibre optics or piezoelectric material as the transducer. Receptor based sensors employ either isolated receptors or intact chemoreceptor structures coupled to electro-transducers as molecular

recognition elements. Receptor based biosensors are not yet practical analytical devices due to problems in sensitivity, reproducibility and difficulty in receptor isolation.

3.1.4 Dipstick and Chromatographic Assays: The principle of operation is that antigen (virus, bacteria or toxin) in a sample is placed on a mounted test strip where it migrates to react with a specific anti-agent antibody in the presence of a suitably coloured, visible indicator. A positive control is usually included and a negative is indicated by lack of colour in the positive window. These formats are capable of high specificity and sensitivity, they are field deployable and inexpensive, have low space and weight requirements and low training requirements and are ideal for field applications.

3.1.5 Electrochemiluminescence: This development in immunoassay technology is available commercially and combines an electrode generated electron transfer event for signal production with immune complex formation on micro-magnetic beads for quick and simple operation. Electrochemiluminescence (ECL) immunoassays can be readily developed in a wide range of different formats and gene probe assays can also be performed by using ECL to detect hybridization of labeled probes to nucleic acid sequences. ECL involves no radioisotopes, the ECL label is a very stable, water soluble compound that can readily conjugate with proteins, haptens, and nucleic acids. Labeled ligands are very stable, with shelf lives of longer than one year in refrigerated storage. Assay development is accelerated by the rapidity, simplicity and versatility of the technique.

3.2 <u>DNA vaccines</u>: This is a new field of vaccination with recombinant DNA vectors encoding antigens and has been termed DNA vaccination or nucleic acid vaccination. If the realization of DNA vaccines equals the promise shown to date, a whole new era of simplified vaccinology may be born. Although there is a precedent for them in live virus vaccines, the same challenges must be overcome as traditional technologies, demonstrating proof of principle, i.e. that the technology can induce the desired and efficacious immune responses and preclinical efficacy, i.e. that the appropriate animal models, protection and adequate duration of protection and immune correlates are seen. Increased emphasis has been placed on determining the mechanisms of antigen presentation and elucidating the immune responses and correlates responsible for protection. Potential advantages of DNA vaccines are the generic nature of the technology and selective augmentation of the immune system. Utilizing identical or similar backbones and plasmid preparation and purification techniques, a wide range of infectious disease and cancer targets can be addressed. This is in contrast to recombinant protein technology where exploration of various host cells is required to find the system where a protein can be properly and optimally expressed. This is then followed by development of process technologies which are unique for each recombinant protein, rather than generic for plasmid DNA regardless of the gene inserted. DNA vaccines promise rapid development and scale-up as compared to protein based vaccines. At this time, significant attention is being placed on the potential safety considerations of this technology although there is probable greater safety than protein based vaccines since there is no risk of anaphylaxis, no side effects due to adjuvants and they are more reproducible than protein vaccines. To date, no mechanism based on unexpected safety limitations have been demonstrated but such safety studies are continuing.

The technology may be very useful for therapy, however, the demonstration of safety must be more stringent before the technology could have any widespread use for prophylactic vaccines.

4.0 Other technological advances

4.1 Phage libraries: In the past five years, phage libraries (binding sequences which can be isolated along with the associated DNA) have become standardized, catalogued and made available through commercial and private exchange. Phage libraries are most useful for rapid development of diagnostic reagents for infectious and/or autoimmune disease, but are also finding application in therapeutics. To increase the affinity of antibodies, the sequence of an immunoglobulin V gene in an antigen-antibody phage antibody is mutated; phage antibodies that bind to the antigen with increased affinity can then be selected from the mutants produced. In this way, antibody affinity has been increased more than a thousandfold. Genetic manipulations make it easy to tailor antibodies to a specific application such as cancer therapy.

4.2 Information networks: The explosive growth of worldwide information networks has been a boon to biotechnology. Protein data banks, DNA libraries and sequences, rapid classification of novel organisms and epidemiological reporting have all gone from low band with exchange among a few academic centres to real time images, text and audio for presentation of genomic sequences, crystallographic structure (fully visualized in three dimensions), and searches of enormous databases for functional characterization of proteins and other biologic macromolecules. Access to these networks is possible through inexpensive commercially available channels at virtually any point on the globe. The World Wide Web, as the global information network is known to most users, probably represents one of the single most important advances in biotechnology.

5.0 Outbreaks of infectious diseases

5.1 As noted in our last report, acquired immune deficiency syndrome (AIDS) continues to represent a newly recognized epidemic illness since the Review Conference in 1991.

5.1.1 AIDS. AIDS has become a major worldwide public health problem. AIDS is caused by human T-cell lymphotrophic virus (HTLV-III), a retrovirus. The disease results from virus infection and destruction of T-helper cells, an important component of the immune system that helps the body ward off disease. Without these cells the patient is susceptible to a wide variety of opportunistic pathogens such as pneumocystis, fungi, and mycobacteria.

5.1.2 AIDS is a classic example of a new disease that has now become pandemic and which arose either from a mutational event of an existing human virus or introduction of an animal (monkey) virus into the human population.

5.2 EBOLA VIRUS. The viral hemorrhagic fevers are a diverse group of human illnesses that are due to RNA viruses from several different viral families, of interest here is the Filoviridae, which consists of Ebola and Marburg viruses. Ebola virus disease was first recognized in the western equatorial province of the Sudan and the nearby region of Zaire in 1976; a second

outbreak occurred in Sudan in 1979. And in 1995 a large outbreak (316 cases) developed in Kikwit, Zaire from a single index case. A related virus was isolated from a group of infected cynomolgus monkeys imported into the United States from the Philippines in 1989. As of yet, this Ebola Reston strain has not been determined as a cause of human disease. The African strains have caused severe disease and death, and it is not known why this disease only appears infrequently or why the most recent strain appears to be less pathogenic in humans. It is unclear how easily these filoviruses can be spread from human to human, but spread definitely occurs by direct contact with infected blood, secretions, organs or semen. The reservoir in nature for these viruses is unknown.

6.0 Summary

As we have attempted to point out in this short paper, since the last Review Conference in 1991, impressive strides have been made in the fields of biotechnology and molecular biology. The confidence expressed in the early years of the Convention that certain technical problems would make biological weapons unattractive for the foreseeable future has eroded. The ease and rapidity of genetic manipulation, the ready availability of a wide variety of production and purification equipment, the proliferation of safety and environmental controls, equipment and health procedures to numerous laboratories and production facilities throughout the world, are all signs of the growing role of biotechnology in the world economy. These same signs unfortunately also give concern for the possibility of misuse of these same technologies to subvert the terms of the Convention. In many ways the recent progress in biotechnology directed to meet public health concerns has also affected the concealment and the ease of production of new potential agents such as toxins and peptides and their delivery systems. Determination of compliance with the Convention, always a difficult task, has been significantly complicated by the new technologies. However, the United States continues to believe that article I, which defines the scope of the Convention, has proved sufficiently comprehensive to have covered the recent scientific and technological developments relevant to the Convention described earlier.

FOURTH REVIEW CONFERENCE OF THE PARTIES TO THE CONVENTION ON THE PROHIBITION OF THE DEVELOPMENT, PRODUCTION AND STOCKPILING OF BACTERIOLOGICAL (BIOLOGICAL) AND TOXIN WEAPONS AND ON THEIR DESTRUCTION

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BACKGROUND PAPER ON NEW SCIENTIFIC AND TECHNOLOGICAL DEVELOPMENTS RELEVANT TO THE CONVENTION ON THE PROHIBITION OF THE DEVELOPMENT, PRODUCTION AND STOCKPILING OF BACTERIOLOGICAL (BIOLOGICAL) AND TOXIN WEAPONS AND ON THEIR DESTRUCTION

Prepared by the Secretariat

1. In paragraph 21 of its report (BWC/CONF.IV/1), the Preparatory Committee for the Fourth Review Conference of the Parties to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction decided to invite States Parties that wished to do so, including the Depositary Governments, to submit to the Secretariat information on new scientific and technological developments relevant to the Convention. This information should cover the applications being made of such developments and their relevance to various aspects of the Convention.

2. The present document contains the information provided by States Parties to the Secretariat, as of 30 October 1996, pursuant to paragraph 21 of the report of the Preparatory Committee.

Cuba

Information for the IV Conference to review the Biological Weapons Convention in connection with the principal scientific and technological developments in Cuba in the past 25 years in the field of microbiology and the areas of veterinary and plant biomedicine and biotechnology - August 1996

The information compiled in this paper cannot be regarded as exhaustive, but reflects in the broadest and most comprehensive manner possible the work accomplished by a group of the most representative institutions in the field in question.

* Re-issued for technical reasons.

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Major results

National Centre for Agricultural Health (CENSA)

* Development of diagnostics for human and veterinary use

* Development of diagnostics for detection of plant pathogens

* Molecular diagnosis of hard-to-detect micro-organisms by conventional means (mycoplasmas, viral diseases of interest in veterinary medicine and bacterial zoonoses)

* Production of vaccines by conventional means and research for the development of recombinant vaccines for veterinary use

* Diagnosis of exotic and quarantinable diseases in animals and plants.

Centre for Genetic Engineering and Biotechnology (CIGB)

* Obtaining, production and use of biological substances: alpha leucocyte and recombinant interferons; recombinant streptokinase; recombinant interleukin 2 (IL-2); recombinant human epidermal growth factor (EGF); transfer factor; human insulin; beta galactosidase enzyme; recombinant human gamma interferon; recombinant protein A; etc.

* Development of technologies for the production of DNA restriction and modification enzymes

* Obtaining of recombinant vaccines against the hepatitis B virus and the tick Boophilus microbius, as well as a natural vaccine against neonatal and post-weaning porcine colibacillosis. Work is under way on possible candidate vaccines against HIV/AIDS

* Obtaining of hybridomas secreting monoclonal antibodies and rat/human hybrid antibodies

* Studies on genetic and immunological characterization of proteins and coatings of micro-organisms

* Development of diagnostics for communicable diseases in humans, animals and plants by conventional methods and by means of molecular biology. Development of new diagnostic studies for SUMA

* Obtaining of transgenic plants and animals (sugar cane, cabbage, potatoes, tobacco, tilapia, rabbits, etc.) and commercial varieties of pest-resistant plants. Technologies for in vitro maturation and fertilization of bovine oocytes

* Studies of infection by hepatitis C, epidemic neuropathy and other diseases

* Production of bioremediators and biological control agents (nematocides).

Centre for Molecular Immunology (CIM)

Obtaining and production of:

* A library of monoclonal antibodies for immunophenotyping of lymphocyte subpopulations

* A library of monoclonal antibodies for immunohistochemistry of tumours

* Injectable monoclonal antibody lor-t3 for prevention and treatment of rejection in organ transplants

* Two monoclonal antibodies for visualization of cancer.

Work is currently under way on new monoclonal antibodies for use in nuclear medicine: to treat lymphomas and lung and breast tumours; recombinant humanized monoclonal antibodies; vaccines for cancer; and basic research on regulation of idiotype networks.

National Centre for Biopreparations (CNB)

Production/research centre which produces 62 types of culture media for fungi and bacteria; recombinant vaccine against hepatitis B (obtained by CIGB); phosphorylation of streptokinase, interferons and albumin; raw material for the biopharmaceutical industry; packaging of products for parenteral use on humans

* Research on introducing new culture media in production processes; development of preparations of allergens for diagnosis and treatment; and development of natural pick-me-ups for human use.

National Centre for Scientific Research (CNIC)

* Use of genetic engineering to develop attenuated strains for immunization against cholera

* DIRAMIC system for rapid detection of urinary infections and the susceptibility of infectious germs to antibiotics.

National Centre for Plant Health (CNSV)

* Use of electron microscopy, serology and molecular biology for diagnosis of phytopathogenic micro-organisms

* Obtaining of antiserums and production of diagnostic kits for plant pathogens

* Development of technologies for the biological production of substances for pest control in cultivated plants

* Use of integrated management techniques for farm pest control

* Development of commercial crops for commercial production of biological substances.

Pedro Kouri Institute of Tropical Medicine (IPK)

* Epidemiological surveillance of exotic communicable diseases. Control measures to prevent their spread in Cuba

* Improvement and automation of the national system for epidemiological surveillance of communicable diseases

* Development of new methods for diagnosis of communicable diseases (amoebas, Fasciola hepatica and dengue). Production of biological reagents and cooperation in the development of new diagnostic kits (SUMA) Introduction of advanced technology (PCR, etc.)

* Participation in preliminary basic research for obtaining viral and bacterial candidate vaccines for humans. Evaluation of new vaccines (against meningitis and hepatitis B). Studies on the impact of vaccines used in the National Vaccination Programme (triple virus - measles, rubella and mumps)

* Research on dengue. Risk factors and technological advances relating to haemorrhagic dengue. Identification of the geographical origin of the Cuban strain of the 1981 epidemic. Basic studies for the purpose of obtaining a vaccine

* Research on tuberculosis and other mycobacterial infections. Resistance to anti-bacillary drugs in Cuba

* Research to confirm that Cuba is free of poliomyelitis. Studies on the circulation of virulent polio virus. National antibody surveys in connection with vaccination

* Studies on HIV infection and comprehensive care for HIV/AIDS patients

* Methods of integral control of vectors of medical significance. Programme for monitoring resistance to insecticides. Biological control of vectors using bacterial and parasitic larvicides and larva-eating fish

* Factors affecting the presence and abundance of river molluscs. Their importance in the epidemiology and control of tropical diseases

* Improvement of the diagnosis and management of acute respiratory infections of viral and bacterial aetiology

* Genetic, biochemical and immunological studies on attenuated strains of Vibrio cholerae.

Pharmaceutical Biological Laboratories (LABIOFAM)

* Production of vaccines against viral diseases in animals: [illegible]; porcine encephalomyocarditis; avian infectious bronchitis; avian encephalomyelitis; Gumboro disease; Newcastle disease; contagious ecthyma; canine distemper and hepatitis

* Production of vaccines against bacterial diseases in animals: erysipelas; bovine brucellosis; bovine bacillary icterohaemoglobinuria; leptospirosis; Neu-S-Suis (Salmonella typhimurium, Salmonella choleraesuis and avian pasteurellosis)

* Development of biological products for vector control: the larvicides "Griseler" (Bacillus sphaericus), "Bactivec" (Bacillus thuringiensis); and the rodenticide "Biorat" (Salmonella enteritidis).

INSTITUTE OF VETERINARY MEDICINE

- Successful completion of the bovine tuberculosis elimination programme

- Vaccination using nationally-produced immunogens to control eastern equine encephalomyelitis, Newcastle disease, symptomatic anthrax and many other diseases which affect animals and in many cases may also affect humans (zoonoses)

- Elimination of bovine brucellosis from more than 90 per cent of livestock, including all the dairy herds

- Efforts to keep the country free of most serious diseases affecting animals

- Development of a vaccine against porcine viral encephalomyocarditis

- Production of biological and other means of diagnosing enzootic diseases in domestic animals.

Finland

Finland has the honour to inform that she has not made any scientific and technological developments relevant to the Convention.

Switzerland

Scientific and Technological Developments
relevant to the BW-Convention

During the last decades biotechnology and genetechnology have revolutionized (and is still doing so) many areas of biological and medical sciences. The possibilities of studying and manipulating genetic information have provided a huge amount of knowledge on basic principles of life. Numerous peaceful applications of this knowledge are employed today worldwide in solving problems of global interest; such as public health and environmental problems.

But soon after the beginning of this biological revolution, it was recognized that these technologies might also have a dark side: their military misuse to create novel Biological Warfare agents.

The possibilities offered by genetic engineering and new biotechnological production capabilities, especially by recombinant DNA (rDNA) and monoclonal antibody (mAb) techniques, to manipulate micro-organisms and parts of them seemed to be almost unlimited. At the beginning of the 1970s BW agents were considered as weapons of little military value, mainly due to their uncontrollability, their unpredictable efficacy and their difficult methods of large-scale production. Although there has been (and still is) a lot of speculation scientists recognized rDNA techniques as ideal means to eliminate these undesirable characteristics of BW agents. As a result many countries have intensified their defensive BW research programmes.

These developments have also been considered with concern and extensively discussed by the States Parties to the BW Convention on the occasion of the Review Conferences. There is a general understanding that the scientific and technological developments relevant to the Convention are covered by the scope of article I of the BW Convention.

Since the last Review Conference in 1991 further advances in science have refined and improved the methods of bio- and genetechnology and some new techniques have been added to the molecular biologist's toolkit, many of them aimed to ease in some way the creation of altered organisms or cells. As offensive BW research is looking for means to alter the characteristics of pathogens in a way that they meet the requirements for militarily suitable biological and toxin weapons, a variety of these techniques have an impact on the development of such warfare agents.

Besides the scientific and technological developments already outlined, as the third Review Conference approaches, progress was made in the bio-production capability such as computer-aided bioreactors with continuous flow systems and integrated biosensors. Today, numerous genetic factors of interest in biological warfare like toxins, resistance and virulence factors, biochemicals and others can be quite easily characterized, isolated and transferred to other host organisms by means of improved recombinant DNA techniques. The polymerase chain reaction (PCR) has developed to one of the most powerful tools for identification, characterization and analysis of genetic material. In the last few years, PCR has been varied and improved continually. Nowadays there is a huge amount of different applications of this method. With the simple and rapid in vitro amplification of genetic material PCR not only offers the possibility of identifying small amounts of specific DNA sequences but is also suited to study DNA from highly pathogenic organisms. By using primers differing at one or several positions from the target sequence it is even possible to modify a known DNA sequence at specific sites. Traditional cloning of DNA relying on technically difficult processes of in vivo replication of the target sequence in a host organism was previously restricted to experienced molecular biologists. The simplification of DNA sequence isolation of PCR has made gene analysis accessible to scientists with less experience in molecular biology.

Biological toxins are increasingly used in medical research and therapy. Consequently there is worldwide an increasing demand for such highly toxic substances. Biotechnology and genetic engineering provide methods for rapid and cheap mass-production of highly potent toxic proteins. The possibility of producing large amounts of toxin within a few weeks resolves the problems arising from stockpiling biological material with relatively low stability, making toxins well-suited as warfare agents.

Besides a possible misuse for developing new biological warfare agents genetic engineering and derivative technologies are extensively employed in research on protective measures against such weapons.

Recombinant DNA techniques are used to develop new and safer vaccines. During the last few years viral vector-based vaccines, split vaccines and synthetic peptide vaccines against numerous diseases have been developed. These may improve the protection not only of a target population against a possible biological attack, but of the aggressors' own troops as well. As a "side-effect" of modern vaccine research, methods may be further developed to insert toxin genes into the genetic material of various viruses.

Highly specific monoclonal antibody fragments can be produced rapidly and in high amounts by new methods (Lerner & Benkovic). These could be used for protection against biological and toxin agents by passive immunization. Monoclonal antibodies coupled with micro-electronics could serve as useful tools in rapid detection and identification of numerous pathogens. Such diagnostic tests based on biosensors are not only a prerequisite for an efficient defence against a biological attack, but they may also be valuable instruments for a future verification of the BW Convention.

These scientific and technological developments are still covered by the scope of article I of the Convention. However, genetically manipulated micro-organisms, unknown viruses, biological toxins, bioregulators and biochemicals still have an increased importance as potential biological warfare agents. Such weapons can be covertly produced and stored on small sites.

The methods of bio- and genetechnology became widespread and are therefore more and more accessible to less experienced scientists, which enhances the danger of proliferation of BW technologies. This danger can be met, among other things, by strengthening the Convention with an effective verification regime.

United Kingdom of Great Britain and Northern Ireland

NEW SCIENTIFIC AND TECHNOLOGICAL DEVELOPMENTS RELEVANT TO THE BIOLOGICAL AND TOXIN WEAPONS CONVENTION

1. Introduction

1.1 Article XII of the Convention on the Proliferation of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction (BTWC) provided for a conference of States parties to

be held to review the operation of the Convention within five years of entry into force. This review was mandated, inter alia to "take into account any new scientific and technological developments relevant to the Convention".

1.2 To assist the work of the First Review Conference in 1980, the Co-depositaries presented a paper on new scientific and technological developments relevant to the BTWC. They prepared separate national papers for the Second Review Conference in 1986. For the Third Review Conference in 1991, papers were prepared by the Depositaries and a number of other States parties. Papers of this type have again been requested by the Preparatory Committee for the Fourth Review Conference due to be held in 1996.

1.3 The previous United Kingdom papers considered advances and changes in science, medicine and industrial applications in the interim since the previous Review Conference, and discussed the implications for scope of the Convention and for the balance between the potential for misuse of science and technology and the potential for improvement in defensive measures. This approach will again be adopted in the present paper, but additionally consideration will be given to any implications for the ongoing work of the Ad Hoc Group mandated with drafting a legally binding instrument to strengthen the Convention.

2. General developments relevant to the BTWC

2.1 There has been a steady increase in the number and scope of biotechnology applications, with better integration of knowledge and techniques contributed from an increasingly wide array of fields such as microbiology, biochemistry, molecular biology, cell biology, immunology, protein engineering, classical breeding, bioprocess technology, and the traditional biotechnology applications of micro-organisms in producing beer, wine, cheese, and other foods. Many commercial and academic projects involve new relationships between the microbiologist, the molecular biologist, and the chemical engineer, and ad hoc relationships are being reinforced by educational programmes such as those under the European BIOTOL (Biotechnology by Open Learning) programme.

2.2 The study of genetics and the application of genetic modification technologies in fields relevant to the BTWC has increased markedly in the five years since the Third Review Conference. Such technologies are being ever more applied in research in the medical and pharmaceutical sector, but there has also been a widening of scope with achievements in the agriculture and food sectors. Some of these advances are reported below. There has been a dramatic increase in knowledge of the genetic basis of pathogenicity of some bacteria and viruses, with genetic sequences available on the internet; and there is an increasingly global dissemination of research techniques that allow cloning of genes including genes for toxin and toxin subunits into a variety of host types - the very large number of successful clonings into micro-organisms is now being augmented with reports of the creation of transgenic animals and higher plants. Should a proliferator adopt a high-tech route for weapons development, the potential range of options has thus increased markedly in this time period.

2.3 Attention to biosafety issues had continued, with increasing development of national regulations covering contained use of micro-organisms and toxic chemicals, and contained use or deliberate release of genetically modified micro-organisms (and other organisms). There has also been increased international cooperation on safety in biotechnology: for example he commitments of Governments under Agenda 21 on sharing experience, capacity-building and international agreement on principles for safety. This commitment has already seen the development of technical guidelines by the United Nations Environmental Programme in 1995.

2.4 Micro-organisms may well be used routinely in the important disarmament context of the Chemical Weapons Convention. Microbial bioremediation is now established as one of the candidate methods for destroying bulk stocks of mustard in national programmes for the destruction of chemical weapons stockpiles. Similar methods may also be applicable to soil contaminated with a range of CW agents and other toxic chemicals.

2.5 Awareness of responsibilities and issues occurring under the aegis of the BTWC, including the work of the Ad Hoc Group in Geneva, has been improved by the information explosion allowed by the internet, which links around 2 million computer systems around the world. The question of whether the internet could have a role in measures included in a future instrument to the Convention needs further consideration.

3. Detection technologies

3.1 R & D into generic and specific detection techniques has continued to address microbiology applications in clinical, food and environmental settings and in biological defence. The possibilities for aerosol characterization have advanced due to the development of multi-parameter characterization systems based on features such as particle size, shape, fluorescence and polarization of light, and a range of commercial equipment is now available. Detection of adenosine triphosphate is used as a generic test for the presence of live organisms in a commercial monitoring device. Flow cytometers have been miniaturized to the point where they are portable; depending on the indicator principles applied, such devices have the potential for generic or specific detection of bacteria. Following the lead of pregnancy test kits developed as hand-held devices and dipsticks, a number of microbial detection techniques based on antibody and gene probe principles have been developed into this type of format.

3.2 Research and development of sampling devices and rapid detection and identification systems for potential BW (biological weapons) agents in a biological defence context is continuing. A range of generic and specific approaches are being considered. Indicator techniques for antibody-antigen reactions include potentiometry, chemiluminescence and fluorescence; other detection principles include laser light scattering in flow cytomerty, and pyrolysis and electrospray mass spectrometry. The latter has recently been used by CBD Porton Down to identify cricket paralysis virus. Information systems to combine data from multiple detector arrays are also being worked on. Biological agent sampling and detection systems were deployed by Allied forces in the 1991 Gulf War.

3.3 The high specificity and sensitivity of specific microbial diagnostic techniques based on PCR (polymerase chain reaction) principles, with the potential to detect as few as 10 micro-organisms, has led to a great increase in the number of research applications, but the potential of PCR technologies in routine diagnosis for example in the clinical laboratory has still to be realized. High cost of reagents, the need for specialized staff for manual techniques and the lack of availability and high cost of automated techniques continue to be factors. However, methods are under development for mycobacteria, HIV, Hepatitis viruses, and Salmonella in foodstuffs. Other nucleic acid amplification methods are also now appearing. Environmental pressures are leading to the development of indicator strategies that do not require the use of radio-labels, but use either absorption or fluorescent dyes. Such approaches are being incorporated increasingly into dipstick formats.

3.4 The above technologies may be worthy of consideration by the Ad Hoc Group in the context of its work on measures that could be included in a future instrument to the Convention.

4. Genetics developments

4.1 It is predicted that the human genome will be sequenced by the year 2005. The information is expected to lead to radical new treatments for a broad range of human disease, but it may also provide information about biochemical pathways that are susceptible to peptides and to other compounds, which could thus be exploited as a hostile act to damage human health. It cannot be ruled out that information from such genetic research could be considered for the design of weapons targeted against specific ethnic or racial groups. However, it is far from clear that the development of such weapons could ever be anything more than a theoretical possibility. Genetic weapons of this type would be a clear contravention of Article 1 of the BTWC.

4.2 Improvement in the speed of automated techniques for high volume DNA sequencing has led to remarkable achievements over the last five years. Many more laboratories have these sequencing capabilities, and are considering total genome sequencing. Genomes from some of the medically important viruses have been sequenced, and three complete bacterial genomes have been published including the pathogen Haemophilus influenzae. Another half dozen or so medically significant Staphylococcus and Streptococcus species are believed to have been sequenced by industry, but these sequences are unlikely to be placed in the public domain. A further 30 or so genomes will probably be sequenced in the near future. Information from genome sequencing has provided insights into the organization of the genome, and this can be expected to lead to a fuller understanding of how it functions. This should allow identification of housekeeping genes and virulence factors, possibly leading to the discovery of new toxins or immunomodulators.

4.3 The polymerase chain reaction has changed molecular biology radically. It has proved invaluable for the cloning and manipulation of natural DNA sequences, and also makes it possible to create new DNA fragments specified by the scientist. This can be done by using PCR in combination with chemical methods to make longer fragments of DNA, which can then be amplified using PCR to make ample amounts for cloning into possible vectors. Unfortunately, this

means that a proliferator now has the potential to clone, say, toxin genes without actually needing the naturally occurring organism as a genetic source. Many more microbes are now amenable to genetic modification either in terms of cloning DNA from them or using them as vectors for carrying and expressing foreign genes.

4.4 Though much of the achievement in the application of genetic modification technologies comes from the improvement of well established methods, new methods continue to appear. An example that may see widespread use within a few years is phage display, in which libraries of filamentous phage expressing random peptide fragments are screened to select molecules with particular binding characteristics. Amplification of the appropriate phage then allows the recovery of the peptide of interest. Screening in vivo has recently been used to find phage that home to specific organs; this approach has the potential for use to identify specific target peptides in tumours or other diseased tissues, allowing the targeted delivery of drugs or of viral vectors in gene therapy.

4.5 Most microbial toxins have been cloned into micro-organisms. Many non-microbial toxins in their natural state are impracticable to produce on any significant scale. However, such are the advances in cloning success rates that at least the protein toxins can now, in principle, be produced on a large scale by growth of a recombinant microbial or eukaryotic cell host using well-established fermentation technology. The genetic sequences of such toxins have been deposited in the public domain molecular biology databases which are growing exponentially. Although in principle there may be an increasing potential for BW proliferators to mix genetic sequences in order to develop hybrid toxin BW agents, the fact that more than 240 bacterial toxins are already known - to say nothing of the snake, spider, and fungal toxins - means that a proliferator would not need to go to such lengths. Application of genetic modification technologies to naturally occurring bacterial toxins can increase production yields in the primary culture medium by 50 fold or more relative to wild type strains, and expression in a different organism can greatly reduce purification problems.

4.6 Much more is now know about the structure-function relationship of various toxin groups. The combination of electrochemical and other biophysical techniques with molecular biology approaches is expected to lead to the resolution of the molecular mechanisms of cell penetration by protein toxins. There is now a substantial amount of research on hybrids of toxins and toxin subunits with antibodies and viruses, often with the long-term objective of specifically destroying diseased cells such as cancer cells. In this type of therapy an antibody-toxin complex would be injected into the blood stream; the antibodies attach to receptors on the target cells which are subsequently eliminated by the action of the toxin, while healthy cells elsewhere in the body are unaffected.

4.7 There have been increasing numbers of reports of the creation of transgenic animals and plants, and for example the genes for ricin have been inserted into tobacco plants. Such achievements may complicate the focusing of declaration modalities under a future instrument designed to strengthen the Convention.

4.8 The applications of genetic modification in research projects with long-term objectives for disease therapy is rising exponentially. In this field there is a general trend away from work with bacteria, principally disabled strains of Escherichia coli, towards eukaryotic viral vectors. As information on human genetics becomes available for example from the Human Genome Project, viruses are being increasingly used as delivery systems to transfer human genes either into mammalian cells in tissue culture or into whole animals as part of gene therapy research. Gene therapy, still in its infancy, involves the insertion of genes into individuals suffering from genetic diseases. One early success story is that the isolation of the genes associated with cystic fibrosis has allowed treatment by delivering wild type DNA through the lungs: this causes reversion to wild type of the alveolar cells, which lasts until a new layer of mutant cells grow up from the underlying germ cells.

4.9 Viral vectors are an attractive starting point for gene therapy because viruses have evolved efficient mechanisms to introduce and express their nucleic acid in recipient cells. The snag is that the host cells have evolved sophisticated mechanisms to rid themselves of viruses. The challenge for gene therapy research has therefore been to achieve efficient and extended expression of the foreign gene while evading the host defences. Two of the more commonly studied delivery systems are retroviruses and adenoviruses, with cancer applications predominating. Genetic modification technologies could be applied to retroviruses, change their tissue tropism and thus overcome the disadvantage that they cannot invade non-dividing cells. In a different context, an example of a successful change in tissue tropism has been publishd for chimeric TBE and Dengue Type 4 viruses.

4.10 The sequencing of complete viral genomes has provided new insights into the molecular basis of virus virulence. The complete genome sequence has been published for four strains of VEE virus and for the attenuated vaccine derivative of one of these strains (TC83); only two mutations were found to be required to convert an infectious cDNA clone of TC83 to full virulence for adult mice. Chimeric viruses have been produced by genetic recombination in order to allow growth of viruses in easily grown cell lines, for production of vaccines or as a strategy for therapeutic drug production. However, chimeric viruses produced by a BW proliferator could circumvent specific detection systems and the immunity acquired by vaccination. A range of viruses that are infectious for man, including vaccinia, adenovirus and VEE virus, have been used as expression vectors to express foreign genes. In principle, a BW proliferator could use such virus systems as vectors for bacterial or other toxins.

5. Vaccines

5.1 The global eradication of smallpox has been sustained, and is thus a testament to the potential of vaccination in disease control. The global eradication of polio is already well advanced and the disease is now rare in the Americas and Western Europe. Changes in economic conditions have led to an increase in vaccine development initiatives in some countries, in many cases based on novel technologies. Practical and ethical problems associated with the human use of vaccines based on recombinant organisms have to date tempered the promised advances in genetically attenuated live vaccines.

However, some recombinant human vaccines have been in use for several years and an example is hepatitis B cloned into yeast - which illustrates the potential production advantage of a recombinant vaccine that is based on a non-pathogenic host and that can thus be produced and handled under low levels of containment. Genetically modified animal vaccines have also started to appear. An animal vaccine that uses capripox virus as a vector to carry genes for Rinderpest virus antigens. This vaccine, which has the advantage of simultaneously protecting cattle against lumpy skin disease and Rinderpest, is currently undergoing trials in Kenya in closed conditions. Another recombinant example is a rabies vaccine for doctoring bait intended to be distributed in the wild as a means of immunizing foxes.

5.2 Recombinant vaccine designs, however, often turn out to be less protective than classical vaccines. There has therefore been increased interest in identifying improved adjuvants, though many candidates trialled to date have proved too reactogenic for general use. Also, increasing importance is now attached to the significance of cellular immunity in vaccine action, particularly in virus infections, as a better means to mimic the real infection. There has been considerable research in the use of new adjuvants, delivery vehicles and immuno-modulators to direct the immune response towards TH1 responses, and one approach is to use toxin-derived molecules. Subunit combination vaccines have been successfully developed, for example based on Diphtheria/Tetanus/acellular Pertussis/Haemophilus influenza type B/hepatitis B, but attempts to increase the number of components tend to be counterproductive because they lead to antigenic overload. A number of vaccines have been formulated for intranasal, oral and slow release delivery. Mucosal vaccination has received increasing consideration, and it is clear that induction of immunity at mucosal surfaces can lead to a disseminated systemic response. These new administration route strategies offer not only logistic advantages for routine use but also the prospect of increased protection against infection by the respiratory route - a factor which could have relevance for biological defence programmes.

5.3 In spite of the efforts being put into vaccine development, effective vaccines are still not available for some important diseases. Thus, current influenza vaccines are still only about 80 per cent effective and duration of immunity is one to two years, shorter than after a natural infection.

5.4 Where the level of disease burden has reduced in a country, it is now recognized that the need for vaccine boosting increases. There has been an increase in the range of serological tests for antibody titres, and in the tests available for functional activity such as bactericidal or virus neutralizing activity, and the trend towards increased emphasis on quality assurance has seen global studies aimed at validating new test methods and standardizing existing methods.

5.5 A major new vaccination prospect that has become firmly established since the Third Review Conference is the use of DNA vaccines. Naked DNA is sued as an inoculum that is taken up by cells where the genes can be expressed for up to two years. The first applications will probably be in vaccines for viral diseases such as influenza and hepatitis A-C, but there is also a massive potential for cancer vaccines. No adjuvants are required, and delivery by injection, transdermal or oral routes is possible. As with other

novel technologies applied to vaccine designs, safety issues will remain high on the agenda. Ethical questions remain about use in healthy humans, and the understandable need to demonstrate the absence of any risk from indirect effects means that progress to clinical trials cannot be hurried. But DNA vaccines at the research stage show promising results in animals, and vaccine development should be much quicker and cheaper than for any genetic modification approach in a microbial vector because one avoids the manufacturing complication of having to express a protein and then carry out downstream processing.

5.6 Because of the trend towards recombinant vaccines and the attention being paid to vaccine efficacy, combined with the strides that have been made in understanding and controlling fermentation processes, the fermanter scale needed for routine vaccine production is reducing fast. Commercially viable operations based on fermanters in the tens of litres range rather than hundreds or thousands of litres will become the norm. Where recombinant vaccines involving safe hosts are produced, low levels of containment will be acceptable. These trends in vaccine manufacture have implications for declaration and compliance monitoring arrangements under the BTWC.

5.7 Some countries have a substantial emphasis on vaccine development, trialling and production in their biological defence programmes.

6. Trends in infectious diseases and their treatment

6.1 There have been important successes in international efforts to alleviate disease burdens. Largely through the activities of international agencies such as the WHO, smallpox has been eradicated. The WHO eradication programme for polio virus by the year 2000 is on target and a programme for measels has started though there is evidence that vaccination may be stimulating antigenic drift among the pathogenic measles viruses in circulation. Great strides have been made in controlling dracunculiasis, leprosy, Chagas disease, neonatal tetanus and onchocerciasis (river blindness). Nevertheless, there is increasing concern about the potential for international spread of disease and about incidents involving emerging and re-emerging diseases and in 1995 the Member States of the World Health Organization called upon the Organization to strengthen and coordinate global surveillance and control of communicable diseases. In response to this resolution, the WHO in October 1995 established the Division of Emerging and other Communicable Diseases, in order to bring together WHO's existing surveillance activities and to promote the development of national and international infrastructure and resources to recognize, monitor and respond to communicable diseases and emerging health problems.

6.2 In spite of the attention placed on hygiene of foodstuffs and water supplies, significant disease outbreaks continue to be reported in developed countries. Particularly problematic are *Escherichia coli* 0157 in water and food and *Cryptosporidium* in water supplies. Antibiotic resistance continues to be a major threat to disease control, and the impact of multiply resistant *Mycobacterium tuberculosis* has only been mitigated by the fact that it appears not to spread very easily.

6.3 The uncontrolled use of antibiotics is implicated in the emergence of the many resistant strains of bacteria which are causing serious problems in hospitals, for example methicillin-resistant Staphylococus aureus. The problem is compounded by the poor discovery record for new antibiotics. The situation may however improve as pharmaceutical companies focus their research on the newly elaborated genome sequences of bacterial pathogens in order to seek new targets for antibacterial drugs effective against multiply resistant strains. The introduction of combinatorial chemistry has greatly increased the number and complexity of compounds that can be generated and screened for therapeutic activity, and it seems that this strategy will replace the random screening of secondary metabolites. Information gained from genome sequencing and from advances in protein engineering and structure analysis may allow rational drug design, but these technologies also raise the spectre of rational design of novel toxic compounds.

6.4 Several newly recognized human viral diseases have emerged within the last few years. Examples that cause fatal human respiratory disease include hantavirus pulmonary syndrome in the United States of America and equine morbillivirus in Australia. Other viruses that have not been detected in humans for several years have re-emerged, for example Ebola and epizootic Venezuelan equine encephalitis. There is evidence that increased contact of humans with vectors has led to new human viral diseases and the rapid spread of established ones, for example the incidence of Dengue associated with the increase in the mosquito vector in Central and South America. Other infectious diseases that are causing concern include Rift Valley Fever, Yellow Fever, Rocio, Hantaan, Sabia, Lassa Fever and plague. In spite of the considerable effort to produce anti-viral drugs effective against important disease agents such as HIV and Herpes virus, clinical trials have been disappointing because these drugs appear to exert a selection pressure for resistant genotypes of the virus. The growing application of molecular biology to anti-viral design is expected to lead to designer targeting of molecules which are essential to the survival of the virus and which become non-functional if they mutate.

6.5 The recent emergence of the prion based disease spongiform encephalopathy in cattle (BSE) and its possible transmission to man to cause a new form of Creutzfeld-Jacob disease (CJD) indicates the potential socio-economic consequences of disease even when it has a very low risk of transmission to humans (only a dozen such CJD cases have been reported to date in the United Kingdom). Infectivity experiments with animals indicate that transmission of this prion requires direct brain inoculation. Although such prion agents are extremely resistant to inactivation and are probably infectious by the aerosol route, they are poor candidates for BW use because of their very low transmission potential and the very long incubation period between exposure and disease. Prions, a malfolded version of a normal cellular protein, clearly fall within the scope of Article 1 of the Convention.

6.6 Research has continued into new compounds active against plant pathogens, but there are still no anti-virus compounds and no good plant bactericides except for antibiotics (which are not approved for crop application in the United Kingdom and are relatively expensive for such use). There is still a lack of fungicides effective against the important wilt

pathogens in the genera Fusarium and Verticillium. Genetic modification of plants to express viral coat proteins and thus confer some protection against infection by the virus or viruses concerned is now relatively commonplace in research but such plants have not yet been released for commercial use in the United Kingdom. Monoclonal antibodies for use in disease detection have been produced with wide and narrow specificity and there are now serological methods for the detection of haptens and plant pesticide residues.

7. Industrial microbiology

7.1 There is a trend towards choosing "workhorse" host organisms for industrial genetic modification applications, as the standard organisms into which to insert genetic material. An example is the established safe organism Escherichia coli K12. This approach offers the advantage of well understood organisms with fast growth and high productivity, that can be used under standardized fermentation techniques. On-line biosensors will lead to better control of processes. All these developments are expected to contribute to higher productivity, thus allowing the use of modestly sized equipment - which has implications for the focus of future declaration and on-site measures under the BTWC.

7.2 Micro-organisms are being used in biotransformation processes to carry out stereo specific chemical transformations on molecules which are the active form in a variety of pharmaceuticals and agrochemicals.

7.3 The application of biotechnology in the manufacture of a greater range of pharmaceutical and high added value products has been accompanied by an increased attention to quality standards and environmental control in some industrial sectors. This has resulted in more widespread use of containment measures designed to protect the product but often having the dual effect of protecting personnel from the process. The growing use of high quality chemical engineering in fermentation processes in all geographical regions of the world also increases the opportunities for misuse of this technology for the production of pathogens or toxins as BW.

7.4 An example of the versatile tools that have been developed for the expression of a variety of heterologous proteins in industry is the baculovirus-insect cell system. Stable baculovirus vectors can be constructed rapidly and with a minimum of viral manipulation. In the last decade, developments in bioreactor design and the use of cell-protective additives have helped to mitigate the sensitivity of the insect cell lines to agitation and sparging and their relatively high oxygen demand. New, serum free media formulations have allowed cost reductions and easier purification of recombinant proteins, which for baculoviruses have included ricin and mammalian derived prion proteins.

7.5 High level protein expression has also been achieved using Semliki Forest virus grown in a range of mammalian cells. The heterologous protein product can constitute up to 25 per cent of total cell protein, and, starting with the target gene, an infectious virus can be obtained in only one to two weeks. Using a viral mutant which is non-infectious allows scale up with good safety control.

7.6 Practical studies to monitor releases in industrial operations have exploited the high sensitivity of PCR techniques. In combination with computational fluid dynamics to determine the trajectory of release, spatial distribution and concentration of cells within a bioprocessing area, PCR has been shown to detect specific genetic material even in the presence of related organisms. The development of biosensors as "artificial noses" that could be used in exit gas streams is becoming feasible.

8. MICROBIAL CONTROL OF PESTS

8.1 Bacillus thuringiensis (Bt) continues to be the most important agent being used to control insect pests. From fundamental studies of the mode of action of these bacteria and the use of advanced genetic modification techniques, Bt toxin genes have been transferred to create transgenic plants. This enables the plant to protect itself from pests by producing an insect-specific Bt toxin. The first major releases of commercial crops based on this principle have occurred, heralding what could turn out to be a major new era in environmentally benign farming avoiding the use of chemicals. The bacterium Bacillus sphaericus, pathogenic for some mosquito species, is another promising biological alternative for pest control.

8.2 The use of viruses to control insect pests has been advanced with the appearance of new commercial pesticides based on the nuclear polyhedrosis virus to control pest species resistant to chemicals. Genetic modification is also being used to improve the performance of these pathogens: new, faster acting strains of virus have been created by incorporating into the genome specific insect toxins derived from other animals such as scorpions. The creation of transgenic plants incorporating genes of insect viruses is also a promising new line of development in crop protection.

8.3 Fungal agents such as Metarhizium spp, and Beauvaria bassiana are being actively developed as biological control agents against important pests such as locusts in Africa and aphids on field crops.

8.4 Advances have been made in developing specific microbes to control noxious weeds such as Rotbellia in South East Asia. The study of the molecular biology of crop diseases like bacterial rots, bacterial wilts, fungal blights and plant viruses has provided the basis to develop the specific diagnostic tests needed by plant propagators to produce disease-free planting material for farmers.

8.5 Of these developments, the direct attack of insects may seem unlikely as a means of biological warfare. However, technologies able to insert genes for viruses or toxins into animals or higher plants could increase the production options for a proliferator. Even entirely legitimate uses of such transgenic plants may create complication for future declaration and inspection measures under the BTWC.

9. CONCLUSIONS

9.1 The three previous papers on new scientific and technological developments prepared by the United kingdom for the earlier review conferences concluded that the rapid pace of scientific and technological developments in

areas relevant to the BTWC demonstrated that implementation of its provisions had not hindered activities for peaceful purposes. The present review of developments up to 1996 indicates that there continues to be no impediment.

9.2 The United Kingdom continues to hold the view that the BTWC fully covers all microbial, other biological agents and toxins, whether naturally occurring or not, including any resulting from the application of genetic modification or other technologies. Technology advances and scientific discoveries over the last five years have not weakened this assessment.

9.3 Each of the earlier papers in this series reported an increased potential for the large-scale production of BW agents, and an increasing technology potential that could be misused to alter candidate-BW agents in order to address shortcomings in their characteristics from a weapons viewpoint or to increase their production potential especially in the case of toxins. Though there has been no quantum technology leap in the intervening five years, the current review indicates that this trend has continued. Increasingly worldwide use of technologies including bioprocessing in the civilian sector have further increased the opportunities for BW proliferation. The markedly increased number and scope of the applications of genetic modification technologies could provide an ever widening range of options for a proliferator choosing a high-tech route for BW development. This could include engineering properties into agents to improve their stability, infectivity, ability to escape the notice of deployed detection and identification systems, or overcome the immunity of target populations.

9.4 There is, however, nothing to indicate that a proliferator having the technical capabilities to adopt a high-tech route is any more likely to do so than formerly, and use of these technologies in an illegal programme will tend to be accompanied by an increased risk of failure and of detection particularly under the operation of a future instrument to the Convention.

9.5 The potential for improving defensive measures against possible BW attack, both in respect of detection and identification techniques and the procurement of improved vaccines, has further improved since the previous Review Conference. The advances in specific identification technologies reviewed in this paper may also be relevant for the measures being considered by the Ad Hoc Group.

9.6 Technolcgy developments such as the reduction in scale and containment levels of vaccine production resulting from the use of more efficient, and recombinant vaccines, and the increasing use of transgenic species including crops, need to be considered in the work of the Ad Hoc Group.

United States of America

TECHNOLOGICAL DEVELOPMENTS OF RELEVANCE TO THE BIOLOGICAL AND TOXIN WEAPONS CONVENTION

1.0 Introduction

1.1 In preparation for the 1996 Review Conference on the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological

(Biological) and Toxin Weapons and on their Destruction (BWC), the Preparatory Committee requested the depositary nations to prepare national papers on new scientific and technological developments relevant to the Convention.

1.2 Since the last Review Conference in 1991, there have been a number of developments and advances in the field of biotechnology, some of which can contribute to improvements in the compliance picture for the BWC. The major breakthroughs have occurred in the fields of analytical developments and vaccinology, both of which have application in the field of medicine and public health as well as industry and agriculture. Of special interest to the BWC are applications in detection and identification which can enhance compliance and production applications which provide further concerns regarding compliance. The number of countries which are developing a biotechnology capability continues to grow as the applications continue to expand into commercial sectors and the resulting industry has expanded in both scope and products developed and marketed. All of these trends continue to have practical significance for the BWC and the problems of compliance with the BWC.

1.3 Our review of technological developments must continue to encompass a broad overview of a wide variety of fields to include basic research through manufacturing in such areas as: biotechnology, molecular biology, medicine, microbiology, biochemical engineering, and pharmaceutical and vaccine production. This activity could include inputs from the academic community, industry, government agencies and departments, trade organizations and similar knowledgeable personnel. The significant advances in assay technology represent positive developments since these can contribute to rapid detection and identification, a critical area in BWC compliance. At the same time the advances made in new, simpler and more rapid production and manufacturing technology remain a BWC concern as well since these developments can contribute to further proliferation. Our concerns expressed in 1991 remain, that while promising great benefits to mankind the advances in technology could be used to produce new substances or modify old ones and lead to a new and significant toxin, biological or biochemical weapons threat and we all must remain aware and cognizant of this potential.

2.0 Advances in industrial application of biotechnology

There are a number of industrial areas where advances in production and assay technology as well as new product technology have relevance to the Convention. These will be detailed in the following sections.

2.1 Modified micro-organisms: Biotechnology allows the development of micro-organisms and subsequent products with new and unique characteristics, many to meet specific purposes. Examples of some of these engineered products are monoclonal antibodies which contain a variety of toxins for therapeutic purposes in treating some leukemias and cancers. Others in various stages of development involve Pseudomonas exotoxin and diphtheria conjugates for cancer and AIDS treatments. In the area of plant pathogens, these same developments are being pursued to produce environmentally safer and more effective pesticides. All of these developments can provide significant benefits to the public health and welfare of society, however, in examining these same developments from the point of view of the BWC, we cannot ignore the potential

misuse of biotechnology to produce new biological agents or improve certain characteristics in those agents already recognized as potential biological agents. Transferring certain genetic characteristics into naturally occurring organisms can potentially create organisms of greater virulence, antibiotic resistance and environmental stability. Changing the microbes genetically could alter their immunogenicity, thereby rendering vaccines and serodiagnostic techniques useless. Otherwise harmless micro-organisms could be altered to produce toxemia or disease, although the host animal would continue to recognize these micro-organisms as innocuous and therefore not defend against them.

2.1.1 Bioengineering of micro-organisms has other implications for the BWC as well. Bacteria and yeasts, genetically altered to produce products, are miniature factories by virtue of their ability to reproduce rapidly. Examples are the production of a wide variety of products by the insertion of genes into tobacco mosaic virus and product production by infection of the tobacco plants with the virus and subsequent product extraction and purification from the plant. Large quantities of compounds, previously available only in minute amounts from natural sources, are now available quickly and easily. Such production methods are now becoming commonplace in industry.

2.1.2 It is now possible to identify genes which have desirable properties, isolate them and transfer them between host organisms. A nearly infinite variety of biological compounds designed for specific uses and given specific characteristics is possible. For example, this should make specific vaccine developments much easier to accomplish. Given the technical progress in this area, future developments should be of concern to the BWC Review Conference. Within the next decade, the potential for misuse of ongoing developments in biotechnology could be most pronounced in the following areas:

2.1.3 Microbial pathogens could be genetically engineered to maximize infectivity and pathogenicity. Likewise they could be modified to increase or decrease their environmental stability and persistency.

2.1.4 Toxins. Naturally occurring protein toxins normally available only from natural sources in small quantities could be made in host organisms by modifying their DNA. Plant and/or fungal toxins could be mass produced. If used as an agent, the origin of these toxins could be difficult to pinpoint, given that they are already present in the environment, albeit at low concentrations. Improvements in biotechnology since the previous Review Conference lead us to believe that production of potent toxins, which until now were available only in minute quantities, and only upon isolation from immense amounts of natural biological materials, can now be produced in kilogram quantities which could be militarily significant.

2.1.5 Peptides. Peptides have been called "the antibiotics of the year 2000" because these biological materials may represent a new class of miracle drugs. Peptides are precursors of proteins made up of amino acids. They are interesting molecules for many reasons. They are active at very low concentrations (one part per billion or trillion) which makes their detection very difficult. They can be successfully modified as agonists (more active products) or antagonists (having a contrary activity). For example,

modification of LHRH, a fertility hormone, by substituting a single amino acid has yielded a product 50 times more potent. Another modification of this same peptide yields a product useful in the treatment of prostate cancer.

2.1.6 Their range of activity covers the entire living system, from mental processes (e.g. endorphins) to many aspects of health such as control of mood, consciousness, temperature control, sleep or emotions, exerting regulatory effects on the body. Even a small imbalance in these natural substances could have serious consequences, inducing fear, fatigue, depression or incapacitation. These substances would be extremely difficult to detect but could cause serious consequences or even death if used improperly.

2.1.7 The predictable modification of peptide and protein structure and function (i.e. protein engineering) is in its infancy. Computer-aided molecular design will rapidly develop, enabling molecules to be manipulated for varying degrees of physiological activity, specificity and stability. Technologies permitting the direct chemical synthesis of peptides and proteins in large yields will, in the more distant future, augment or replace microbial production of these molecules.

2.2 Advances in production. As mentioned earlier, once a suitable recombinant organism has been engineered, exploiting it becomes a matter of using established procedures. Biological production technology has proceeded to the point where large quantities of biological products can be produced quickly in small facilities. An example is the production of bovine growth hormone, a compound developed to increase milk production. Two genetically altered pesticides are about to be approved for commercial use which represent a "second generation" of biopesticides which will not degrade quickly in the environment due to a microencapsulation process. Long-term refrigerated storage, in some cases will not be needed because large quantities can be produced very quickly starting from a minute seed stock. Several relevant technological considerations regarding biological production are discussed below.

2.2.1 Mammalian cell culture. Recent advances in mammalian cell culture make possible the growth of mammalian cells on the surface of minute beads, rather than on the inner surface of glass roller bottles. These cell culture systems provide the ideal environment for the growth of viruses. The new technique greatly simplifies virus production and allows large-scale yields in facilities of modest size. A further advantage of these cell culture systems is the ease of purification and concentration of the virus produced since it is relatively clean and free of impurities from the production process in contrast to earlier fertile egg production systems. As another example of advances in this field, the amount of tissue culture media needed to produce antibodies has been reduced a hundredfold by the use of encapsulated hybridomas. Such developments are eroding the distinction between production facilities and small laboratories.

2.2.2 Continuous flow fermentors. The introduction of computer controlled, continuous flow fermentors has dramatically increased productivity. Most likely the size of fermentors operating by batch process can be reduced a thousandfold by conversion to a continuous flow process.

2.2.3 Hollow-technology fibre. Hollow fibre technology provides an example of the industrial production potential of the new technologies. This technology permits a far greater concentration of cells with a markedly increased rate of recovery in a shorter time than previously obtained using roller bottles. This equipment occupies less than one twentieth the volume of the previous technology. Concentration and purification of a wide variety of protein substances can now be accomplished in large-scale liquid chromatography columns, yielding pure, highly fractionated proteins. In the isolation of such cellular biomaterials as pyrogens, a similar transformation has taken place. Separation and reconstitution of the product can now be accomplished in about an hour using new compact ultrafiltration methods, whereas older methods took as much as four days.

2.2.4 Safety and environmental standards. Pharmaceutical plants around the world increasingly have incorporated safety and environmental release provisions akin to those which were once unique to BW production facilities for product purity, worker safety and environmental and community protection, making it increasingly difficult to distinguish between permitted and prohibited activities.

2.2.5 Improvements in equipment, speed of production and quality of product are a common occurrence in the history of the commercial development of any new technology. Many oil utilizing micro-organisms produce a surface active compound which can emulsify oil in water and facilitate recovery of the oil. A microbial glycolipid emulsifier has been produced in large quantities and shown to act as a powerful dispersant of oil in water. Unlike chemical surfactants, it is non-toxic and biodegradable. Other strains of micro-organisms have been developed which have the potential for use in biodegradation of chemical nerve agents, mustard, explosives and hazardous wastes such as PCBs. Other commercial developments in the area of agricultural research using biotechnology have occurred in crop research. Cereals, forages, fibre crops and fruits and vegetables that are highly adapted to environmental stresses such as drought, cold, heat and toxic soil minerals are being developed.

2.2.6 In animal research, scientists are developing effective diagnostic tests for diseases of beef and dairy cattle, pigs, sheep, chickens and turkeys, and producing new and more reliable preventative measures against livestock diseases. Other researchers are using the tools of biotechnology to grow bacteria that can break down toxic wastes. Because of the large number of technical innovations in the area of industrial microbiology, it has become more difficult to assess compliance with the BWC since its signature in 1972. Developments intended to increase production, decrease cost and create safer conditions for handling biological materials have blurred former distinctions important for purposes of assessing compliance - for example, between a large production facility and a laboratory. Also, capabilities to break out of the Convention in a very short time have increased.

3.0 Advances in analytical and vaccine technology

Though not without constraints, developing biological and toxin weapons is a much easier task than developing adequate defences against them.

However, the very advancements in biotechnology that have caused increased concern have also put new tools in the hands of those conducting permitted biological research.

3.1 Developments in assay technology: There are a number of developments now available for rapid clinical diagnosis of a variety of potential biological agents.

3.1.1 Enzyme-linked immunosorbent assay (ELISA): This is one of the more widely used assay techniques today. The ELISA assay is based on the interaction of an antibody with its specific antigen and the subsequent detection and assay of this interaction by an enzymatic reaction. ELISA has three steps: first immunoreactants are immobilized on a solid phase to act as capture immunosorbent, second the sample to be captured is added, and third immunochemical reactions are detected by adding specific enzyme conjugated antibodies directed against the sample. ELISA assays are rapid and easy to perform. They are highly sensitive, specific and reproducible. Equipment is relatively cheap. The immunoreagents have a long shelf life when stored frozen, no hazardous radioisotopes are required and large numbers of samples can be assayed simultaneously.

3.1.2 Nucleic acid probe detection: Another effective approach is the use of nucleic acid probe detection which is based on the ability of labeled ribonucleic or deoxyribonucleic acid (RNA or DNA) probes to recognize and bind to complementary target nucleic acid sequences in a sequence-specific manner, through the process of molecular hybridization. The use of nucleic acid hybridization technology has enormous potential in rapid diagnosis which is enhanced through the use of the polymerase chain reaction (PCR) which allows for the amplification of minute quantities of genomic material without extensive purification. The specificity depends upon the stability of the probe-target hybrid formed under the hybridization conditions used such as pH, temperature, salt concentration and probe length, the sensitivity also depends on the hybridization conditions of temperature, time, probe material and the detection system used to detect the labeled probe. Nucleic acid probe detection systems are highly specific and depending upon the probe and the primers used can detect discrete genes. They do require highly trained personnel to perform the assay, contamination of the sample must be prevented, cost may be prohibitive and the test will generally only detect agents with known, unmodified gene sequences.

3.1.3 Biosensors: Biosensors evolved from the combination of two disciplines: modern biotechnology and advanced electronics. Biosensors have advantages over conventional detectors including high sensitivity, capacity for miniaturization and the availability of multiple measurement sites. Rapid, individual analysis can be expedited, often by minimally trained personnel. Three major types of biosensors exist: biocatalytic, immunochemical and receptor based. Biocatalytic are generally based on purified enzymes immobilized on a solid substrate combined with a signal transducer. The immunological biosensors are based on coupling immunological interactions between antibodies and antigens with electrochemical assays which can encompass fibre optics or piezoelectric material as the transducer. Receptor based sensors employ either isolated receptors or intact chemoreceptor structures coupled to electro-transducers as molecular

recognition elements. Receptor based biosensors are not yet practical analytical devices due to problems in sensitivity, reproducibility and difficulty in receptor isolation.

3.1.4 Dipstick and Chromatographic Assays: The principle of operation is that antigen (virus, bacteria or toxin) in a sample is placed on a mounted test strip where it migrates to react with a specific anti-agent antibody in the presence of a suitably coloured, visible indicator. A positive control is usually included and a negative is indicated by lack of colour in the positive window. These formats are capable of high specificity and sensitivity, they are field deployable and inexpensive, have low space and weight requirements and low training requirements and are ideal for field applications.

3.1.5 Electrochemiluminescence: This development in immunoassay technology is available commercially and combines an electrode generated electron transfer event for signal production with immune complex formation on micro-magnetic beads for quick and simple operation. Electrochemiluminescence (ECL) immunoassays can be readily developed in a wide range of different formats and gene probe assays can also be performed by using ECL to detect hybridization of labeled probes to nucleic acid sequences. ECL involves no radioisotopes, the ECL label is a very stable, water soluble compound that can readily conjugate with proteins, haptens, and nucleic acids. Labeled ligands are very stable, with shelf lives of longer than one year in refrigerated storage. Assay development is accelerated by the rapidity, simplicity and versatility of the technique.

3.2 DNA vaccines: This is a new field of vaccination with recombinant DNA vectors encoding antigens and has been termed DNA vaccination or nucleic acid vaccination. If the realization of DNA vaccines equals the promise shown to date, a whole new era of simplified vaccinology may be born. Although there is a precedent for them in live virus vaccines, the same challenges must be overcome as traditional technologies, demonstrating proof of principle, i.e. that the technology can induce the desired and efficacious immune responses and preclinical efficacy, i.e. that the appropriate animal models, protection and adequate duration of protection and immune correlates are seen. Increased emphasis has been placed on determining the mechanisms of antigen presentation and elucidating the immune responses and correlates responsible for protection. Potential advantages of DNA vaccines are the generic nature of the technology and selective augmentation of the immune system. Utilizing identical or similar backbones and plasmid preparation and purification techniques, a wide range of infectious disease and cancer targets can be addressed. This is in contrast to recombinant protein technology where exploration of various host cells is required to find the system where a protein can be properly and optimally expressed. This is then followed by development of process technologies which are unique for each recombinant protein, rather than generic for plasmid DNA regardless of the gene inserted. DNA vaccines promise rapid development and scale-up as compared to protein based vaccines. At this time, significant attention is being placed on the potential safety considerations of this technology although there is probable greater safety than protein based vaccines since there is no risk of anaphylaxis, no side effects due to adjuvants and they are more reproducible than protein vaccines. To date, no mechanism based on unexpected safety limitations have been demonstrated but such safety studies are continuing.

The technology may be very useful for therapy, however, the demonstration of safety must be more stringent before the technology could have any widespread use for prophylactic vaccines.

4.0 Other technological advances

4.1 Phage libraries: In the past five years, phage libraries (binding sequences which can be isolated along with the associated DNA) have become standardized, catalogued and made available through commercial and private exchange. Phage libraries are most useful for rapid development of diagnostic reagents for infectious and/or autoimmune disease, but are also finding application in therapeutics. To increase the affinity of antibodies, the sequence of an immunoglobulin V gene in an antigen-antibody phage antibody is mutated; phage antibodies that bind to the antigen with increased affinity can then be selected from the mutants produced. In this way, antibody affinity has been increased more than a thousandfold. Genetic manipulations make it easy to tailor antibodies to a specific application such as cancer therapy.

4.2 Information networks: The explosive growth of worldwide information networks has been a boon to biotechnology. Protein data banks, DNA libraries and sequences, rapid classification of novel organisms and epidemiological reporting have all gone from low band with exchange among a few academic centres to real time images, text and audio for presentation of genomic sequences, crystallographic structure (fully visualized in three dimensions), and searches of enormous databases for functional characterization of proteins and other biologic macromolecules. Access to these networks is possible through inexpensive commercially available channels at virtually any point on the globe. The World Wide Web, as the global information network is known to most users, probably represents one of the single most important advances in biotechnology.

5.0 Outbreaks of infectious diseases

5.1 As noted in our last report, acquired immune deficiency syndrome (AIDS) continues to represent a newly recognized epidemic illness since the Review Conference in 1991.

5.1.1 AIDS. AIDS has become a major worldwide public health problem. AIDS is caused by human T-cell lymphotrophic virus (HTLV-III), a retrovirus. The disease results from virus infection and destruction of T-helper cells, an important component of the immune system that helps the body ward off disease. Without these cells the patient is susceptible to a wide variety of opportunistic pathogens such as pneumocystis, fungi, and mycobacteria.

5.1.2 AIDS is a classic example of a new disease that has now become pandemic and which arose either from a mutational event of an existing human virus or introduction of an animal (monkey) virus into the human population.

5.2 EBOLA VIRUS. The viral hemorrhagic fevers are a diverse group of human illnesses that are due to RNA viruses from several different viral families, of interest here is the Filoviridae, which consists of Ebola and Marburg viruses. Ebola virus disease was first recognized in the western equatorial province of the Sudan and the nearby region of Zaire in 1976; a second

outbreak occurred in Sudan in 1979. And in 1995 a large outbreak (316 cases) developed in Kikwit, Zaire from a single index case. A related virus was isolated from a group of infected cynomolgus monkeys imported into the United States from the Philippines in 1989. As of yet, this Ebola Reston strain has not been determined as a cause of human disease. The African strains have caused severe disease and death, and it is not known why this disease only appears infrequently or why the most recent strain appears to be less pathogenic in humans. It is unclear how easily these filoviruses can be spread from human to human, but spread definitely occurs by direct contact with infected blood, secretions, organs or semen. The reservoir in nature for these viruses is unknown.

6.0 Summary

As we have attempted to point out in this short paper, since the last Review Conference in 1991, impressive strides have been made in the fields of biotechnology and molecular biology. The confidence expressed in the early years of the Convention that certain technical problems would make biological weapons unattractive for the foreseeable future has eroded. The ease and rapidity of genetic manipulation, the ready availability of a wide variety of production and purification equipment, the proliferation of safety and environmental controls, equipment and health procedures to numerous laboratories and production facilities throughout the world, are all signs of the growing role of biotechnology in the world economy. These same signs unfortunately also give concern for the possibility of misuse of these same technologies to subvert the terms of the Convention. In many ways the recent progress in biotechnology directed to meet public health concerns has also affected the concealment and the ease of production of new potential agents such as toxins and peptides and their delivery systems. Determination of compliance with the Convention, always a difficult task, has been significantly complicated by the new technologies. However, the United States continues to believe that article I, which defines the scope of the Convention, has proved sufficiently comprehensive to have covered the recent scientific and technological developments relevant to the Convention described earlier.